

NUTRIENT MANAGEMENT STRATEGIES FOR ALLEVIATION OF DROUGHT STRESS IN WHEAT (*Triticum aestivum* L.)

By

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Dedicated to my

Family Members

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ABSTRACT

Water-limited conditions in early growth stages negatively affect germination and seedling growth, often leading to suboptimal plant population and poor stand establishment. Germination and seedling growth of ten local wheat (*Triticum aestivum* L.) genotypes in response to induced water stress conditions and supplemental foliar fertilisation with macronutrients (NPK) were investigated. In two laboratory experiments, the observed germination parameters and calculated stress indices were used as screening criteria for drought tolerance. In one glasshouse experiment, the dose and combinations of N, P, and K for foliar spray were optimised. Germination parameters viz. germination percentage, germination index, promptness index, and germination stress tolerance index declined in response to the increasing polyethylene glycol induced stress levels. Water stress conditions imposed by withholding irrigation at seedling stage reduced plant height stress tolerance index and dry matter stress tolerance index but increased root length stress tolerance index and root to shoot ratio. Based on the results of germination attributes and stress indices, Bhakkar-02 was the most drought tolerant genotype and Shafaq-06 was the most drought sensitive genotype among all tested genotypes. Supplemental foliar fertilisation of macronutrients (N, P, and K), alone or in different combinations improved the water relations, gas exchange characteristics and nutrient contents in both contrasting genotypes, Bhakkar-02 and Shafaq-06. Foliar spray NPK in combination was the most effective treatment in improving plant growth under both well-watered and water-deficit conditions. Subsequently, the best combination of foliar NPK was tested in wire house and field conditions to evaluate the most appropriate growth stage for supplemental fertilisation. In wire house experiment foliar application of NPK spray improved the water relations, gas exchange characteristics (i.e. through accumulation of soluble sugars), total free amino acid, and proline. The antioxidant activity was also improved with foliar NPK spray at anthesis stage. In field experiments foliar application of NPK in combination improved the number of grains per spikelet and 1000-grain weight, which ultimately increased the grain yield at anthesis stage in normally irrigated plants as well as under water stress conditions at anthesis stage in both wheat genotypes. The water shortage at anthesis stage decreased the yield and its components more severely as compared to tillering stage. The drought tolerant Bhakkar-02 performed well under water stress. So, foliar application of NPK at anthesis stage under water stress conditions gave better results as compared to tillering stage.

Chapter-1

INTRODUCTION

Agriculture is the backbone of Pakistan. From the day of independence Pakistan was primarily agriculture based country. But with the passage of time Pakistan turned into more diversified country as industrialization took hold. But the development of industries had not abolished the survival of agriculture in Pakistan even though the stake of agriculture had declined significantly since from the day of independence. The role of agriculture cannot be denied in the economic development of Pakistan. Pakistan is still considered as an agricultural country (Sheikh et al., 2012).

Wheat is very important for nutritional point of view as it earns a highly important spot among the other crop species being broadly grown as a staple food sources. The prominence of wheat is primarily due to the fact that its seed can be crushed into flour, semolina, etc., which makes the basic constituents of bread and other bakery products like inorganic ions pastas. Most of the world's population get main source of nutrients from wheat (Šramková et al., 2009).

Wheat grain generally contains protein, starch, carbohydrate and fats. Carbohydrates are present in wheat grain from 60-90%. The protein content of wheat grains may vary between 10% - 18% of the total dry matter. The amount of starch contained in a wheat grain may vary between 60%-75% of total dry weight of the grain. Lipids are present only in a small extent in cereals (1.5-2%), but they have a significant effect on the quality and the texture of foods. Wheat grain also contains vitamins 1% and 1.2-2% (Belderok et al., 2000).

The production of wheat in the world was 690 million tones in 2012 which is 6% higher than 2011 (FAO, 2012). Wheat is the major staple food item of Pakistan and leading grain crop of the country. Pakistan accounts for about 3.7 % of the total area under wheat cultivation in the world. It contributes 10.1 percent to the value added in agriculture and 2.2 percent to GDP of Pakistan. Wheat was cultivated on an area of 8.693 million hectares, showing increase of 0.5 percent over last year's area of 8.650 million hectares. The production of wheat crop was 24.23 million tons which is 3.2 percent more

than last year crop. National average of wheat is 2.787 t/ha (Government of Pakistan, 2012-13).

The availability of water to crops is decreasing day by day in Pakistan. The water availability during Rabi season this year was expected 31.9 MAF which was 12.4 percent less than normal availability (Government of Pakistan, 2012-13). The availability of water for plant growth is considered to be one of the most significant agricultural troubles. Plants during their life cycle frequently faced the periods of soil and atmospheric water deficit and the shortage of water is likely to raise in upcoming days (Chaves *et al.*, 2002). Drought is the only most significant threat to the food safety. Supply of world's water is restraining. For rapidly increasing population demands of future food supply is likely to further intensify the effects of drought (Somerville and Briscoe, 2001). Drought stress reduces germination and seedling stand (Kaya *et al.*, 2006). Plants established growth through cell division, cell enlargement and differentiation, and also involves complex interface of genetic, physiological, ecological and morphological events and their complex interactions. Events which are affected by drought determine the quality and quantity of plant growth. Reduction in cell growth due to reduction in turgor pressure is the most sensitive physiological process for drought. (Taiz and Zeiger, 2006). Decrease in rubisco activity, reduces photosynthesis under severe drought stress. (Bota *et al.*, 2004). Dehydration consequences in cell shrinkage and as a result decline in cellular volume that makes cellular contents further viscous. Consequently, enhance the chance of protein-protein interaction, leads to their aggregation and denaturation (Hoekstra *et al.*, 2001). Increased in viscosity of cytoplasm by increase in concentration of solutes and may be harmful for the execution of enzymes, together with those of the photosynthetic equipment (Hoekstra *et al.*, 2001).

Significant losses have been reported in wheat grain yield due to drought stress but it is conditional on the developmental stages at which crop plant suffer with stress. The intensity of the reaction depends on the stress severity and its time period, as well as the plant developmental stage. Wheat crop requires water for the complete growth period, but there are certain phases, which are more susceptible to water scarcity, and any water deficiency during this stage can result in substantial yield losses (Bukhat, 2005).

Environmental stresses like drought affects water and nutrient supply to the plants thus affecting adversely plant development and yield (Erdem *et al.*, 2001). Plant growth and development is very crucial under drought stress which is closely related to absorption and consumption of nutrients like nitrogen, phosphorus and potassium.

Drought stress result in decreased in nutrient uptake particularly of phosphorus and nitrogen which may contribute towards yield loss. In addition, even though the crop can penetrate the roots into deeper and wetter parts of the soil profile and also nutrients concentrated in dry soil can be unavailable to plants (Wright, 1982).

Optimum amount of water and nutrients supply is necessary for adequate crop production. It has been reported that negative effects of water stress on crop growth and development diminished by nitrogen application. It also affects the photosynthetic capacity by increasing the thylakoid and stomatal proteins in leaves (Makino *et al.*, 1992; Bungard *et al.*, 1997). It has been reported that nitrogen is the essential part of the RNA, DNA, chlorophyll, Amino acid and in numerous enzymes plays an essential role in the cell metabolism. Nitrogen under low water stress increase the grain yield under low nitrogen application in winter wheat though beneath drought high dose of nitrogen prove to be detrimental (Nielson and Halvorson, 1991). Application of nitrogen minimized the negative effects of drought in pearl millet dry matter and grain yield production (Ashraf *et al.*, 2001).

Application of Phosphorus fertilization considerably progress plant growth under water stress (Ackerson, 1985; Studer, 1993; Garg *et al.*, 2004). Phosphorus cause the optimistic effect on plant growth by attributing to enhance in stomatal conductance under drought stress (Bruck *et al.*, 2000). Phosphorus being a component of nucleic acid, phospho-proteins, phospholipids, dinucleotides and adenosine triphosphate is necessary for storage and transfer of energy processes, photosynthesis, some enzyme regulation and transport of carbohydrates (Hu and Schmidhalter, 2001). Phosphorus treated plant show higher cell membrane stability, water relation and water use efficiency (Sawwan *et al.*, 2000).

Potassium fertilization mitigate the negative impacts of drought on plant growth (Anderson *et al.*, 1992; Studer, 1993; Sangakkara, 2001), by increasing energy status, stomatal regulation, osmoregulation, protein synthesis, charge balance and homeostatis (Beringer and Trolldenier, 1978). Potassium reduces rate of transpiration under drought stress (Anderson *et al.*, 1992). Potassium plays an important role by maintaining the high pH in the stroma and it is against the oxidative damage to chloroplast (Cakmark, 1997). Potassium regulates stomatal functioning (Kant and Kafkafi, 2002), which shows the improvement in crop growth and yield under low water status (Umar and Moinuddin, 2002). Among the nutrients like potassium, foliar application only cannot provide a considerable amount of the crop need. Therefore, the additional application of potassium

as foliar fertilization to soil-applied fertilization of these nutrients is significant in condition where nutrient supply through soil is restricted. The application of foliar potassium in many crop species at or near the time when supply of these nutrients either becomes deficient or needed the most, has gained popularity (Weir, 1998).

Decrease in uptake of nutrients under drought stress needs to be improved in time to avoid yield losses. Application of nutrients through soil might be too late and less beneficial. In this condition, additional methods of fertilizer application can be potentially effective such as water run, split side dress and foliar spray

. A reasonable research work on improving use efficiency of soil-applied nitrogen, phosphorus and potassium under drought stress has been reported in the literature; however, very little information is available on the synergetic effects of foliar and soil-applied nitrogen, phosphorus and potassium in alleviating the adverse effects of drought stress.

Keeping in view these aspects the present study is planned with the following objectives:

- i. To investigate the effect of water stress on various physiological and biochemical attributes of wheat.
- ii. To assess the effect of exogenously applied Nitrogen, Phosphorus and Potassium on growth, yield, physiological and biochemical traits of wheat grown under drought conditions.
- iii. To determine optimum combination of supplemental foliar applied Nitrogen, Phosphorus, and Potassium for improving drought tolerance and yield of wheat.

Chapter-2

REVIEW OF LITERATURE

2.1. Germination and seedling growth

The effects of drought on yield of crops depend on their severity and the stage of plant growth during which they occur. Seed germination is the first stage of growth that is sensitive to water deficit. Therefore, seed germination, vigor are rudiments for the success of stand establishment of crop plants. Germination becomes limited due to low moisture. Time of maturity and crop yield are determined by rate and degree of seedling establishment (Rauf *et al.*, 2007).

Polyethylene glycol (PEG), a drought inducing chemical, is frequently used in the medium to screen out drought tolerant varieties at early stage of seedlings. PEG could be used to change the osmotic potential of the solution and it creates the water stress condition. PEG can be used to modify the osmotic potential of nutrient solution culture and thus induce plant water deficit in a relatively measured way (Lagerwerff *et al.*, 1961; Zhu *et al.*, 1997). The molecular weight of PEG molecules is 6000 (PEG 6000) which are sluggish, non-ionic and effectively impervious that may often been used to maintain constant water potential throughout experiment periods without physiological damage (Lu and Neumann, 1998). Molecules of PEG 6000 are too small to influence the osmotic potential but these molecules are large enough that cannot be absorbed by plant and not even estimated to enter intact plant tissues promptly (Carpita *et al.*, 1979). Water is withdrawn from the cell because PEG does not enter into the apoplast. Therefore, PEG solution mimics dry soil more closely than solutions of low Osmotica, which infiltrate the cell wall with solutes (Veslues *et al.*, 1998).

2.2. Plant water status

Relative water content is a significant property of plant leaves that is directly proportional to soil water content (Sarker *et al.*, 1999). It is reported as a significant indicator of drought stress in plant leaves (Merah, 2001). Exposure of stress to plants

causes the decrease of relative water content of leaves which also lower the water potential; and osmotic potential (Grover *et al.*, 2004). Osmotic adjustment i.e. the active lowering of osmotic potential in response to water stress is regarded as the mechanism which significantly contributes to increase water stress resistance (Khan *et al.*, 1993). Usually, reduction of leaf water potential occurs with the intensity of stress (Ashraf *et al.*, 1992; Galle *et al.*, 2002). Leaf water potential is also measured to be a dependable parameter for estimating the response of plant to water stress. Significant differences in water potential of wheat genotypes observed under water stress (Singh *et al.* 1990). However Sinclair and Ludlow, (1985) reported that relative water content (RWC) of leaves was a better indicator of water status than water potential. Jiang and Huang, (2002) reported that decrease in leaf relative water contents due to water stress. It is familiar from the various studies that the relative water content and water potential of the cells reduced as drought stress imposed (Lawlor and Cornic, 2002). Under these circumstances stomata play crucial role in the optimization of transpiration and photosynthesis. Stomatal limitation under drought stress limits photosynthesis which can be attributing to reduced intercellular CO₂ concentration (Lawlor and Cornic, 2002). Reduction in leaf water potential during water stress condition, genotypes shows lower water potential are thought to be drought tolerant (Ashraf and O'Leary, 1996).

Drought is the most limiting factor of crop productivity. In Pakistan more than 5.0 million ha of the total cropped area is rain fed, which is about 22% of the total cultivable land (Khalil and Jan, 2002). Drought is a multipart physicochemical process, in which many biological macro molecules and small molecules are involved, such as deoxy ribose nucleic acid, ribose nucleic acid, proteins, carbohydrates, lipids, hormones, ions, free radicals, mineral elements (Apel and Hirt, 2004; Casati *et al.*, 2004). It is the best choice to develop drought tolerant varieties for the crop production and yield improvement under water deficit conditions (Siddique *et al.*, 2000). To develop new varieties there is a great importance of physiological and biochemical approaches to know the complex responses of plants to water deficiency. Under water deficit condition accumulation of some compatible solutes such as sugars, betaines and proline which adjust the intercellular osmotic potential and these solutes do not interfere plant metabolic reactions. It is reported that water stress causes the oxidative stress due to which lipid peroxidation increase and ultimately cause membrane injury and pigment bleaching (Sairam and Saxena, 2000).

Accumulation of proline under water stress condition in plants could only be useful for possible drought injury sensor despite of its role in stress tolerance mechanism (Zlatev and Stoyanov, 2005). Under water deficit condition proline accumulation occurs and that has a role in tolerance mechanism of plants against oxidative stress and that is found to be a main approach to avoid damaging effects of water stress (Vendruscolo *et al.*, 2007). Moaveni, (2011) reported that wheat is the key food for more than 35% of world population and wheat production is primarily limited to zones which are facing shortage of water. This study explores the variations of proline accumulation, relative water content, membrane stability, chlorophyll, protein and (MDA) Malon Di Aldehyde of 4 wheat Varieties at flowering stage under water stress condition. Drought stress increases the accumulation of proline, membrane stability and MDA while decrease of relative water content, chlorophyll content and protein content compared to control.

Inadequate supply of water is a key abiotic factor that adversely affects the production of crops worldwide (Waraich *et al.*, 2011). In many ways drought stress affects the growth and physiology of plants (Waraich *et al.*, 2011). The effects of drought on yield of crops depend on their severity and the stage of plant growth during which they occur. Plants have adapted a number of mechanisms to provide long lasting protection against drought stress by leaf waxiness, and trichome density which can reduce the water lost through stomata (Save *et al.*, 2000). Plants close their stomata under water limiting condition to reduce their transpiration rate which is a process mediated by abscisic acid, a signaling molecule in the plant roots. Low amount of water loss through transpiration slows down the production of reactive oxygen species (Monneveux *et al.*, 2006), while these reactive oxygen species can cause DNA nicking, amino acid and protein oxidation, and lipid peroxidation (Reddy *et al.*, 2004), and it also disrupts the lipid bilayer structure and decreases the function of membrane-bound enzymes (Miller *et al.*, 2010). Reduction in turgor pressure and cell growth occurs when severe drought stress prevails (Steudle, 2000). There are also many molecular changes occur in plants that enable them to survive better under short term water deficit and permit them for quick changes in short time.

The mechanism of osmotic adjustment has been shown in number of cereals (e.g. barley (Gonzalez *et al.*, 2008) and maize (Hajlaoui *et al.*, 2010), and it can be contribute to photosynthetic and stomatal adjustment mechanisms in these crops. Osmotic adjustment in plants contributed by many compounds like mannitol, sorbitol, proline and quaternary ammonium compounds as glycine betain (Bartels and Phillips, 2010). These

compounds also surround the hydration shell around delicate proteins and prevent their degradation under osmotic stress (Galinski, 1993).

2.3. Stomatal conductance and transpiration

Water stress inhibited the photosynthesis which is the basic phenomenon to influence the crop productivity (Chaves and Oliveira, 2004). Closure of stomata occurs under water deficit condition which also leads to reduction in transpiration and intercellular concentration of CO₂ that also prevents the Calvin-cycle at moderate water stress (Horton *et al.*, 1996). Water stress decreased the photosynthesis that can be attribute to both stomatal and non-stomatal limitations (Shangguan *et al.*, 1999). Closure of stomata controls the water loss that has been recognized as an initial response of plant to water stress under field conditions leading to limited uptake of CO₂ (Cornic and Massacci, 1996). Cornic, (2000) reported that closure of stomata generally believed that main factor of reduced photosynthesis under water limited condition. Maroco *et al.*, (1997) reported that decline in leaf turgor pressure or low water potential or low humid atmosphere caused the stomatal closure. Opening and closing of stomata are more closely related to soil moisture than to leaf water status. This proposes that dehydration of roots produced a chemical called ABA that is responsible for stomatal closure (Davies and Zhang, 1991). Strong time dependence in stomatal reaction to air humidity and water status was also establish (Franks *et al.*, 1997), proposing that certain diurnal variations in stomatal function may consequence from metabolic events with a circadian rhythm (Chaves *et al.*, 2002).

Reduction in relative water content (RWC) has been well-known to persuade stomatal closure and consequently a similar reduction in photosynthetic rate (Cornic, 2000). Therefore, a high degree of co-regulation occurs between stomatal opening and photosynthesis (Hubbard *et al.*, 2001). Rahbarian *et al.*, (2011) studied two drought tolerant and two drought sensitive genotypes under 100% field capacity and water stress conditions. They assessed growth, photosynthesis, chlorophyll fluorescence, chlorophyll contents and water relations in the seedling, early flowering and podding stages. Shoot dry weight, transpiration rate (E), CO₂ assimilation rate (A), and PSII photochemical efficiency (F_v/F_m) in all genotypes decreased significantly under water stress conditions. PSII photochemical efficiency was higher in tolerant genotypes than in sensitive genotypes in seedling and podding stages. In all stages water use efficiency and CO₂ assimilation rate were also high in tolerant genotypes under water stress. So, water use

efficiency, photosynthetic rate and PSII photochemical efficiency could be effective markers for studies of tolerance to drought stress.

2.4. Biochemical changes during water stress

2.4.1. Chlorophyll and carotenoids

Most essential pigments active in the photosynthesis are chlorophyll a and b. In photosynthesis, leaf chloroplast contains antenna pigments which absorb solar radiation and through resonance transmission the subsequent excitation is channeled to the pigments of reaction center, which release electrons and as a result the photochemical process set in motion. The chlorophylls, Chla and Chlb, are the most essential of these pigments, and are therefore practically essential for the oxygenic conversion of light energy to the stored chemical energy which is the source of energy for the atmosphere. From a physiological perception, leaf Chl content (for example, how it differs together between and within species) is consequently a parameter of important concern in its own right. Carotenoid which is an accessory pigment, also have a very significant part in photosynthesis. Carotenoids synthesis is a genetic character in plants, but environmental circumstances also play a substantial role (Bojovic and Stojanovic, 2005).

Severe water stress restricts the process of photosynthesis in plants by initiating the changes in chlorophyll and its components through damaging the photosynthetic apparatus (IturbeOrmaetxe *et al.*, 1998). Water stress reduced the leaf chlorophyll contents of plants (Ommen *et al.*, 1999). Manivannan *et al.*, (2007) reported decrease in total chlorophyll contents including chlorophyll a content, the chlorophyll b content under water stress conditions in all sunflower varieties. Reactive oxygen species caused the reduction in chlorophyll contents under drought stress. (Smirnoff, 1995).

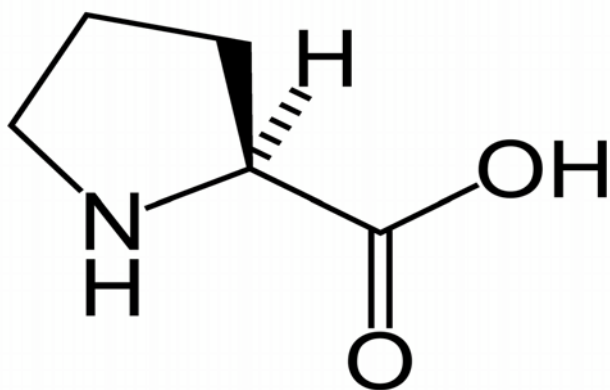
2.4.2. Osmoregulation

Osmoregulation is the active regulation of the osmotic pressure of cell to maintain the homeostasis of the cell's water content; that is it keeps the cytoplasm from becoming too dilute or too concentrated (Ramanjulu and Bartels, 2002). These solutes are also mentioned as osmoprotectants due to their capability to defend the cellular components from dehydration damage (Rhodes and Hanson, 1993). Free proline, particularly, is supposed to play a crucial role in cytoplasmic tolerance in several species and, consequently, responsible for the tolerance of entire plant under severe drought stress (Barker *et al.*, 1993). This stress-related adaptive mechanism of osmoregulation imparts protection to dehydrating tissues (Morgan, 1992).

These solutes are highly soluble in water and these are uncharged molecules which is the major properties of these solutes (Ballantyne and Chamberlin, 1994). Furthermore, at high concentrations these molecules have much low or negligible harmful influence on macromolecule-solvent interactions (Yancey, 1994). Some of the inorganic ions which protects the proteins from unfolding because proteins should be remain in folding to perform functions, so these inorganic ions enter in the hydration sphere of proteins easily and favoring unfolding while compatible osmolytes tend to be excluded from the hydration sphere of proteins and help in stabilizing the folded protein structures (Low, 1985). Wyn jones *et al.* (1977) reported that compatible solutes play important role in cytoplasmic adjustment of plants under osmotic stress. Moinuddin, and Khanna-Chopra, (2004) reported that plants become more tolerant to drought stress when limited water is available, under such situation that favor the osmotic adjustment.

2.4.2.1. Proline

Proline accumulation occurs in higher plants in large quantity in response to environmental stresses (Hsu *et al.*, 2003; Kavi Kishore *et al.*, 2005). Accumulation of proline helps the plant to maintain cell turgor, proline is the major osmolytes which produced more faster than other amino acid in plants under drought stress (Valentovic *et al.*, 2006). Therefore, production of proline in plants can be used as a criterion for drought stress resistance assessment for varieties (Gunes *et al.*, 2008).



2.1. Fig. Structure of Proline

In many plant species a correlation has been found between proline accumulation and stress tolerance. The quantity of proline usually greater in stress tolerant plant than in stress sensitive (Ashraf and Foolad, 2007). Proline accumulation is reconciled by both ABA dependent and ABA-independent signaling pathways (Zhu, 2002). Great amount of proline permit a plant to keep low water potentials. Accumulation of compatible solutes involved in osmoregulation through lower water potential, permits further water to be taken up from the atmosphere, therefore protecting the instant effect of water scarcity within the organism (Kumar *et al.*, 2003). Proline acts as osmolytes and also play crucial role in stabilizing the cellular structures such as membranes and proteins, buffering cellular redox potential and scavenging free radicals under stress conditions, alleviating cytoplasmic acidosis, and retaining proper NADP⁺/NADPH ratios compatible with metabolism (Hare and Cress, 1997). It can also functions as a protein compatible hydrotrope (Srinivas and Balasubramanian, 1995). Furthermore, upon relief from stress quick catabolism of proline may provide adequate reducing agents that support mitochondrial oxidative phosphorylation and production of ATP for rescue from stress and repairing damages induced by stress (Hare and Cress, 1997; Hare *et al.*, 1998). Additionally, proline causes the induction of stress responsive genes that keep responsive elements of proline (e.g. PRE, ACTCAT), in their promoters (Chinnusamy *et al.*, 2005). Cell membrane and proteins are protected by proline against the negative effects of high concentration of inorganic ions and temperature extremes (Santarius, 1992; Santoro *et al.*, 1992). The main site where proline is stored in reaction to drought stress or salinity stress is cytosol in plant cell (Ketchum *et al.*, 1991). Apical meristems of maize root is the main localized site where accumulation of proline occur in response to water stress which includes better proline deposition to the growing region, and seems to be need of abscisic acid (Ober and Sharp, 1994; Sharp *et al.*, 1994). Osmotic stress triggers the accumulation of ABA in plants which is believed that control the expression of genes that is involved in proline biosynthesis (Xiong *et al.*, 2001).

2.4.2.2. Sugars

Photosynthesis causes the production of sugars which are the substrates of carbon and energy metabolism and that leads to the production of polysaccharides like starch and cellulose in plants so these are necessary for plant growth and development (Gupta and Kaur, 2005). Major changes in carbohydrate metabolism occur during water, cold and salinity stress (Kaur *et al.*, 2000). Under abiotic stress sugars play an important part throughout plant growth and development by regulating carbohydrate

metabolism. Stimulation of a huge amount of stress responsive genes by glucose has also been stated, showing the part of sugars in environmental reactions (Price *et al.*, 2004).

Kameli and Loesel (1993) reported increase of soluble sugars in the leaves of wheat under water stress. These sugars are also considered to play a significant part in osmotic adjustment which is broadly regarded as adaptive response to drought stress conditions (Kameli and Loesel, 1995). Khan and Naqvi, (2012) reported that drought tolerant species have more accumulation of reducing sugars while drought sensitive have less. So on the basis of reducing sugar; it could be useful for the selection of drought tolerant species. Limited water can affect a marked decrease in sucrose, fructose and glucose content of grains of sensitive varieties (Saeedipour, 2011). Kerepesi and Galiba (2000) stated that drought tolerant cultivars accumulated additional sucrose than sensitive ones. Increase of soluble sugars due to different environmental stress in different parts of plants. (Prado *et al.*, 2000; Gill *et al.*, 2001). In the situation of salt (Gill and Singh, 1985) and water stress (Prado *et al.*, 2000), due to alteration of these stresses has been indorsed to the stress-induced proliferation in sugar levels.

2.4.3. Protein

Cheng *et al.*, (1993) reported that water stress causes the alterations in protein expression, accumulation and synthesis during plant growth has been observed in numerous plant species. Kottapalli *et al.*, (2009) repoted that quantitative and qualitative changes in proteins occur due to water stress. Modulations in proteins depend on the nature of plant species and type of tissue under water stress (Terri *et al.*, 1986). Decrease in protein contents under drought stress condition (Singh and Usha, 2003), but there is increase in total soluble protein contents in the roots and shoot of barley plant seedlings as the concentration (El-Tayeb, 2005).

Several specific proteins have been characterized in water stress plants during the past two decades, which can be classified as Late embryogenesis abundant (LEA), responsive to ABA, dissication stress protein, dehydrins (dehydration-induced proteins), proteases, cold regulation proteins, enzymes necessary for the biosynthesis of numerous osmoprotectants (proline, sugars and glycinebetain.), the anti-oxidative enzymes (catalase CAT, superoxide dismutase SOD, ascorbate peroxidase (APX), glutathione reductase GR, peroxidase POD) and protein factors that have been involved in the regulation of signal transduction and gene expression, such as protein kinases and transcription factors (Tzvi *et al.*, 2000). Ashraf *et al.* (2003) reported that proteins

accumulation in leaves of the plants under drought stress is considered as an adaptation mechanism. Inquiries to regulate the water stress induced modifications in the proteins would found the purposeful relevance of these proteins for evolving water tolerant cultivars (Katam *et al.*, 2007).

2.5. Water stress and oxidative damage

Decrease in activity of photosystem II (PSII) consequences in difference among the generation and utilization of electrons, apparently subsequent in changes in quantum yield. Due to the change in quantum yield, the photochemistry of chloroplasts in the leaves of water stressed plants change that result in the dissipation of additional light energy in the PSII core and antenna, therefore, producing reactive oxygen species which are possibly hazardous under water stress conditions (Peltzer *et al.*, 2002). The change in the photosystem activities and the inhibition of CO₂ assimilation, beside with the changes in photosynthetic electron transport capacity that results in enhanced production of active oxygen via the chloroplast Mehler reaction (Asada, 1999). Therefore, increased in the production of superoxide, hydrogen peroxide and singlet oxygen due to increased in the rate of O₂ photo-reduction in the chloroplast under water stress condition (Robinson and Bunce, 2000). Free radicals have very damaging effects on the plant's cell structure like lipid peroxidation, DNA nicking, amino acid and protein oxidation (Johnson *et al.*, 2003).

Most biological macromolecules are most sensitive to reactive oxygen species; these ROS damage them to impair their function. The injured objects are improved by repair or by replacement via de novo biosynthesis. However, under extreme stressful circumstances and due to highly injured target particles, a catastrophic cascade of events set in, subsequent in cell death. Initiation of oxidative stress in water-stressed plants is well recognized (Ramachandra-Reddy *et al.*, 2000; Chaitanya *et al.*, 2002; Mano, 2002). In fact, it has been stated that considerable damage to plants affected by exposure to numerous stresses that is connected with oxidative damage at the cellular level (Allen, 1995). The fortune of cells under stressful atmospheres is determined by the period of stress as well as the defensive ability of the plant. Reactive oxygen species plays a critical role in affecting cellular injury under water stress. As the production of reactive oxygen species increased, it causes induction of the genes expression to synthesize antioxidant. These antioxidants detoxify the reactive oxygen species causes the tolerance of plant against water stress conditions (Mano, 2002).

2.5.1. Antioxidants

Water stress increased the activity of antioxidant like catalase, peroxidase and ascorbate peroxidase (Salekjalali *et al.*, 2012). Larson, (1988) reported that plants have endogenous systems to defend cellular and subcellular systems from the cytotoxic effects of these active oxy-free radicals. All plant have the mechanisms of reactive oxygen species to detoxify them and it can be classified into as non-enzymatic (anthocyanins, ascorbic acid, carotenoids and flavanones enzymatic (catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), glutathione reductase (GR). Cellular concentration of O₂ and H₂O was regulated by superoxide dismutase. These further, break down by peroxidase and catalase. During medium stress conditions, theses radicals are effectively detoxify by this antioxidant system. But in dissication sensitive plant, under severe water stress, the rate of free radical production become more and the injury is compulsory (Mundree *et al.*, 2002).

The degree to which the activities of antioxidant enzymes and the quantity of antioxidants increase during drought stress is exceptionally variable between numerous plant species and even among the two cultivars of the same species. The level of reaction depends on the species, developmental stage and the metabolic state of the plant, as well as the length and intensity of the stress. Several stress circumstances cause an increase in the entire foliar antioxidant activity (Pastori *et al.*, 2000), however slightly is identified about the coordinative control of activity and appearance of the diverse antioxidant enzymes in plant cells that are exposed to water stress.

2.6. Effect of water stress on yield

The interaction of genotype and environment are essential contributors of variation in determination of yield and yield components. Kernel weight is great influence by the position of kernel on the spike. Great differences in kernel /spikelet and kernel weight specified that these two variables are accountable for yield variations to stress through spikelet and kernel growth phase (Duggan and Fowler, 2006). Baser *et al.* (2004) reported the consequence of drought stress on the yield and yield components of winter wheat and found a reduction of about 40% in yield under water deficient situations as linked to control. The selection of high yielding varieties frequently based on weight of grains/spike as stated by numerous investigators as the utmost closely related variable linked to grain yield per unit area (Kumbhar *et al.*,

1983). Variation in wheat grain yield for 1000-grain weight is 20% which also showed as the major yield component (Collaku, 1989). Drought sensitive plants of wheat decrease yield more than the drought tolerant plants during water stress conditions (Gáspár *et al.*, 2005).

Significant reduction in wheat grain yield had been shown due to water stress reliant on the developmental stages at which crop plant experienced stress. Number of kernels per ear and number of heads decreased due to water stress before anthesis in wheat (Dencic *et al.*, 2000; Guttieri *et al.*, 2001). While drought stress imposed at later stages might additionally cause a decline in number of kernels per ear and kernel weight (Baque *et al.*, 2006; Saeedipour, 2011). The capacity of wheat cultivars to perform practically better in variable rainfall and drought stressed environments is a significant trait for consistent production under water stress conditions (Pirayvatlov, 2001). But, it is considered that water stress is usually less detrimental to grain yield when occurring early in the crop cycle (Blum, 1996). Before anthesis water stress can decrease number of ear head and number of kernels per ear (Mary *et al.*, 2001). Though drought stress executed during later stages might, moreover, cause a decline in number of kernels/ears and kernel weight (Gupta *et al.*, 2001). Wheat is more susceptible to drought stress from stem elongation to heading and from heading to milking (Zhang and Oweis, 1999).

Abayomi and wright, (1999) conducted two experiments to assess the effects of drought stress applied at different growth stages, on the growth, grain yield and its components of spring-wheat (*Triticum aestivum* L.) cultivars. The effects of water stress on growth and yield were greater when the stress was applied in the late vegetative and post-anthesis stages as compared to early vegetative stage where recovery was better. Number of tillers decreased with water stress during early vegetative stage, but this was compensated by production of late tillers after rewatering. Water stress during late growth stages decreased spikelet fertility, and the number of grains per ear which was the maximum contributor to the decline in yield. Other components, though, such as number of ears per unit area also contributed to the decrease in yield. Generally, drought stress had a larger effect on yield when it happened in the reproductive stage and it is recommended that screening for drought stress tolerant genotypes in cereals must be done at the reproductive stage. Akram, (2011) conducted an experiment to determine the sensitivity of wheat to water stress and changes in water relations and yield of wheat (*Triticum aestivum* L.) under water stress conditions applied at different growth stages. They had applied four water stress treatments on two wheat cultivars, which were

sustained by withholding water at tillering, anthesis, and at both stages. Drought stress caused decline water potential, osmotic potential, and turgor potential, in leaf relative water contents, growth and yield components of both the wheat cultivars. Increased in yield and yield components were associated with high value of relative water contents. Successive stresses at both growth stages initiated severe decrease in yield and yield components in both cultivars of wheat.

2.7. Nutrients and water stress

2.7.1. Nitrogen

Plants require nitrogen in large amount but nitrogen use efficiency always remains low in dry land regions, due to which nutritional deficiency occur which is a major constraint for high crop yields and enhancing crop quality. Under water stress condition nitrogen application may improve drought tolerance of plant to enhance yield (Chipman *et al.*, 2001; Li, 2007). Yang *et al.*, (2000) studied the application of nitrogen under moderate water stress. They reported that nitrogen application may improve remobilization from stored carbohydrates in vegetative organs to grain during grain filling stage, which may encourage the synthesis of starch and formation of grain yield during post anthesis drought. Several studies showed the advantageous role of nitrogen on modulation of water status and nitrogen metabolism under drought by promoting nitrate reductase activity and accretion of osmotic nitrides such as free proline, glycinebetain and soluble protein under water stress (Saneoka *et al.*, 2004; Zhang *et al.*, 2007; Monreal *et al.*, 2007). Nitrogen nutrition plays a significant role in biosynthesis of nitride reductase in plants which is involved in nitrogen metabolism (Li, 2007).

Xie *et al.*, (2011) reported that nitrogen application considerably increased maize photosynthetic rate, resulting in improved shoot biomass production. NPK application significantly altered Cu and Pb mobility in plants. Although, Cu and Pb concentration much increased by nitrogen application but, its concentration was significantly decreased under phosphorus application. Kharel *et al.*, (2011) reported that nitrogen increased the water use efficiency and reduced yield loss of wheat. Shangguan *et al.*, (2000) investigate gas exchange and water use efficiency to N nutrition in winter wheat under well-watered and drought conditions. Nitrogen nutrition remarkably improves photosynthetic gas exchange parameters of winter wheat under both water conditions.

2.7.2. Phosphorus

Phosphorus is a necessary element that is required for carbohydrate transport and enzymatic production of photosynthesis (Alam, 1999; Raghothama, 1999). Water deficit induced low cytoplasmic inorganic phosphorus can limit crop productivity by reducing the triose phosphorus exchange rate between chloroplast and cytosol (Pieters *et al.*, 2001) while this can be improved at the pollination stage where high amount of carbohydrates required for the reproductive growth and thus for yield (Westgate and Boyer, 1986). Waraich *et al.*, 2011 reported that phosphorus increase the root growth of plant and thus enhance the water use efficiency. Noack *et al.* (2010) also reported that phosphorus required by the dry land cereal crops for the early root growth and tillering.

According to Bundy *et al.* (2001), the quantity of plant-available phosphorus in some soils has improved considerably over the past 25 years due to phosphorus fertiliser and manure application in addition of crops needs. The fact that solution phosphorus fertilisers were not as simply available to crop producers in the past, also donated to the customary application of phosphorus to the soil. Leach and Hamielec, (2001) detected a substantial increase in both cob index and starch content when phosphorus was applied at four-leaf growth stage.

2.7.3. Potassium

Potassium is required by plants in much larger quantity than that of all the other soil supplied nutrients (Tisdale *et al.*, 1985). Potassium plays vital role in plants and stimulates biological processes as in enzymatic activity, respiration, photosynthesis, chlorophyll contents, carbohydrate synthesis, water balance in leaves as well as stomatal regulation and direct effect on disease resistance (Mesbah, 2009).

Welch and Flannery, (1985) reported that increasing potassium supply increased water use efficiency of corn plants. Abu-Grab and Sanna, (1999) mentioned that potassium was more significant of stressed corn plants than well – irrigation ones. Plant response to environmental stress like drought depends on its nutritional status, for the improvement of plant to drought stress; application of potassium seems to have positive effects in overcoming drought stress. Application of K alleviates the negative effects of water stress in plants by increasing translocation and sustaining water equilibrium within plants (Greenwood and Karpinets, 1997).

Potassium insufficient plant's seeds are shriveled, small and more susceptible to disease, so optimum amount of potassium can improved seed quality (Fusheng, 2006). Seed composition influenced by application of NPK that can effect development of

embryo and consequently seed vigor (Sharma and Anderson, 2003). Baque *et al.* (2006) reported that dry matter accumulation significantly affected by water stress in leaf, root and stem which was due to lower uptake of nitrogen, phosphorus and potassium under water stress condition and most of characters in plant which contribute to grain yield were affected considerably. Application of potassium in high amount increased the uptake of nitrogen, phosphorus and potassium particularly under water stress conditions. Therefore, potassium application might alleviate the adverse effects of water stress on wheat yield.

2.8. Foliar nutrient application

2.8.1. Nitrogen

Oosterhuis and Bondada, (2001) reported that foliar applications have also been found significant to meet late season nitrogen requirements of crops and to reload nitrogen in the leaves. Though, supplemental foliar nitrogen fertilisation as a source to soil applied nitrogen is a mainly effective method and alone foliar application cannot meet the considerable magnitudes of the crop's needs specifically of macronutrients like nitrogen. There are numerous studies on improved crop yields with foliar nitrogen fertilisation such as wheat (Smith *et al.*, 1987) and soybean (Garcia and Hanway, 1976). Foliar application of a nutrient may in fact encourage root absorption of the same nutrient (Oosterhuis, 1998) or other nutrients through improving root growth and increasing nutrients uptake (El-Fouly and El-Sayed, 1997). Foliar application of nitrogen increased nitrogen contents in plants of chick pea at flowering and 50% flowering (Palta *et al.*, 2005).

Foliar application of nitrogen in the form of urea (1%) and micronutrients (Fe, Zn, Mn) enhanced growth and yield of wheat. Yaseen *et al.* (2010) reported that supplemental foliar application of nitrogen (1% urea) showed considerable increase in nitrogen concentration, uptake, protein contents and 1000- grain weight as compared with control. It also exposed that spraying wheat plant with (1% urea) showed noticeable increment in micronutrients concentration and uptake. Gooding and Davies (1992) revealed that the application of foliar nitrogen fertiliser at anthesis was the best effective fertiliser at increasing grain protein concentration (GPC). Additionally, a positive association between N, provided by foliar fertilisation, and Tea *et al.*, (2007) observed the bread-making quality of flour, while yield responses to foliar nitrogen fertiliser have varied considerably and yield has only increased when previous N applications to the soil had been sub-optimal (Readman *et al.*, 1997). Increasing the rate of nitrogen fertiliser significantly increased the yield and its components of wheat (Sharshar *et al.*, 2000;

Sushila and Gajendra, 2000). Amal *et al.* (2011) reported that foliar fertilisation of urea and potassium initiated significant stimulatory effect on growth parameters, though; foliar feeding with urea 2% + K₂O 2% gave the maximum significant values for all growth characters and also for yield and its components. Chlorophyll synthesis increased with foliar applied nitrogen (Suwanarit and Sestapukdee, 1989).

Khan *et al.* (2009) stated that plant height, spike length, hundred grains weight, biological yield, grain yield significantly increased by foliar application of urea. For all processes protein synthesis nitrogen is the fundamental ingredients. Foliar fertilisation of nitrogen increased the crop yield of wheat (Smith *et al.*, 1987) and soybean (Garcia and Hanway, 1976). Jairo *et al.* (2005) demonstrated the increase in yield and seed protein contents of chick pea (*Cicer arietinum* L.) by foliar application of urea under drought stress. Sen and Chalk, (1996) described that percentage of foliar-absorbed nitrogen and root or shoot dry matter in wheat and sunflower was correlated only when plants were nitrogen deficient. Application of 50, 50, and 50 kg N, P, and K ha⁻¹ as basal dose and foliar spraying of 2 % urea (27.5 kg N ha⁻¹) at different growth stages increased growth and yield of rice i.e. tillering, panicle initiation and grain-filling stage under rainfed conditions has also been described by Badole and Narkhede, (1999). Increased in yield due to higher number of pods and significant decline in flower and pod by foliar applications of urea at first flowering, full flowering and pod setting was recorded in soybean (Okon *et al.*, 2003). Foliar application of nitrogen increased the leaf area, boll number, boll dry weight and yield in cotton by late season (Bondada *et al.*, 1999).

Different forms of foliar applied nitrogen-phosphorus-potassium (NPK) fertilisers increased the leaf area, fresh and dry weight and yield in corn and sweet corn (Ling and Silberbush, 2002). Foliar application of nitrogen on wheat increased the plant height, number of tillers plant⁻¹, straw yield and economic yield (Shaaban, 2001), spike weight; stem weight (Sabahi and Rahimian, 2000). Foliar application of urea compared with soil applied nitrogen (urea) caused more than 10% increased in biomass (Bieluga *et al.*, 1997). Optimum nitrogen management and to minimize nitrogen losses to the environment urea can be applied to the plants as foliar application (Haverkort and MacKerron, 2000). Though, urea concentration is critical for its foliar application.

Sousa *et al.*, (1996) suggested that urea concentration for foliar sprays should not exceed than 5% (W/V). They studied the effect of nitrogen fertiliser with different urea concentration (1, 5, 10 or 20%) and observed that 10 and 20% urea spray decreased the total dry matter possibly as a consequence of phytotoxicity in bean. Foliar urea initiated

the complications of leaf scorching in winter wheat however; it can be overcome by modifying the number of sprays applied (Kettlewell and Juggins, 1992). Foliar application of 2% urea with and without basal application of NPK on transplanted rice (Sye-75) in rain fed conditions resulted in significant increase in growth and yield at tillering, panicle initiation and grain filling stage with a basal dose (50, 50 and 50 kg/ha) of NPK (Badole and Narkhede 1999). Sharma and Jain, (2003) reported the effect of foliar sprays of different agrochemicals (thiourea, zinc sulphate, urea and boric acid) and stated an increase in growth, yield, harvest index and oil content of Indian mustard. Yildirim *et al.* (2007) demonstrate the effect of foliar urea (0.4, 0.8 and 1.0%) on broccoli cultivars (AG 3317 and AG 3324) and detected increase in heads size and weight with 0.8 and 1.0% urea. Increase in dry matter (DM), relative water content (RWC) and nitrate reductase activity (NRA) by foliar nitrogen under water stress in maize (Zhang *et al.*, 2009).

2.8.2. Phosphorus

Foliar application of Phosphorus under mild water deficit gave higher seed dry weight per plant and the water use efficiency was higher on phosphorus treated plants (dos-Santos *et al.*, 2004). Very little study has been conducted to evaluate the use of foliar phosphorus fertilisation. Hardly any work has been done to evaluate the relative efficiency of soil-applied versus foliar-applied phosphorus fertilisers. Initial work by Wittwer and Teubner, (1959) indicated that plants obtain water, gases and an extensive range of solutes from the environment through the foliage. Substantial amount of investigation has been completed to detect the factors affecting foliar nutrient uptake (Swanson and Whitney, 1953; Fisher and Walker, 1955; Koontz and Biddulph, 1957). It has been noted that at low pH levels (pH 2 to 3) foliar-applied phosphate solution generally is taken up much faster (Wittwer and Teubner, 1959). Higher rate of mono-ammonium phosphates are absorbed at lower pH values (Wittwer *et al.*, 1957).

As recommended by Mosali *et al.* (2006), foliar fertiliser use efficiency should be much higher; meanwhile, various conceivable pathways for phosphorus loss related with the application of nutrient to the soil are abolished. Instead, the nutrient is directly “fed” to the plant, and the accessible phosphorus is eagerly taken up, translocated and consumed. So, considerable smaller amounts of fertiliser would be adequate to fulfill crop nutritional necessities and to efficiently correct phosphorus deficiency mid-season. As indicated by Mosali *et al.* (2006), for several decades the prospective of foliar phosphorus application has been undervalued due to usually poorer levels of phosphorus in soils.

Today, though, much greater phosphorus concentrations are present in many cropping system as a consequence of application of phosphorus fertiliser in addition of crops needs.

Phosphorus deficiency in corn, if occurs in mid-season then it would be corrected by foliar phosphorus fertilisation. This would allow the crop with the phosphorus additional needs to accomplish higher grain yield as well as increase phosphorus use efficiency (PUE). The effectiveness of phosphorus fertiliser might be greater if phosphorus is applied foliar compared to soil applied phosphorus fertiliser. Foliar application of nutrients like nitrogen (N), P, and potassium (K) increase the yield and quality of many crops (Römheld and El-Fouly, 1999). In a pot culture corn trial, Barel and Black, (1979) observed that foliar spray of ammonium triple-phosphate to the mature leaves of corn and 66% of phosphorus applied was absorbed within 10 days and 87% of the absorbed amount was translocated, displaying that corn plants were positive and competent in uptake and consumption of foliar phosphorus applied.

Harder *et al.* (1982) detected a substantial decrease in corn grain yields when foliar phosphorus was applied 2 weeks after silking. Sawyer and Barker, (1999) assessed the influence of foliar mono-potassium phosphate and urea fertiliser on corn grain yield and grain components. They found no effect of foliar fertilisation on corn grain yield and grain characteristics. The accomplished results can be described by the subsequent: grain yield levels were relatively high at the assessed sites; soils did not obtain any phosphorus fertiliser pre-plant due to very high soil phosphorus levels. Consequently, the crop, most probably, did not experience any phosphorus deficiencies and hence did not show any response to phosphorus fertiliser. The consumption of foliar-applied phosphorus fertiliser has been found to be dependent on nutrient availability of phosphorus in the soil for both peanuts (Halevy *et al.*, 1987) and cotton (Halevy and Markovitz, 1988). As proposed by Benbella and Paulsen (1998), foliar phosphorus should be applied to the crop later in the growing season to efficiently delay leaf senescence. According to the findings by Mosali *et al.* (2006) timing of foliar phosphorus application is very important and also suggests that foliar application of phosphorus in mid-season extend the grain filling stage and, consequently, increase yield potential. Ling and Silberbush, (2002) matched the effectiveness of foliar fertilisation to that of the soil-applied fertiliser. They assessed the effect of application of numerous forms of nitrogen–phosphorus–potassium (NPK) fertilisers and concluded that foliar fertilisation may be used as a supplement to compensate for the inadequate uptake of nutrients by the roots from the soil applied

fertiliser. For instance one nutrient may improve or constrain the absorption of another nutrient when applied together.

Several positive effects of foliar phosphorus fertiliser application in corn (Leach and Hamielec, 2001), and (Thavaprakash *et al.*, 2006), wheat (Sherchand and Paulsen, 1985), (Batten *et al.*, 1986), (Haloi, 1980), and barley (Qaseem *et al.*, 1978) have been reported. Sherchand and Paulsen, (1985) and Batten *et al.*, (1986) stated that foliar application of KH_2PO_4 resulted in higher grain yield in winter wheat coupled with the delay in leaf senescence in hot and dry growing conditions. Qaseem *et al.* (1978) attained higher yields when phosphorus fertiliser was applied to barley as a foliar spray solution. Mosali *et al.* (2006) reported wheat grain yield to be poorly connected with phosphorus concentration. They observed that delayed maturity is one of the key benefits of foliar phosphorus application in wheat production systems. The best results were accomplished when pre-plant phosphorus was attached with mid-season foliar phosphorus fertilisation. Pongsakul and Ratanarat, (1999) described that foliar application of NPK fertilisers improved grain yield of both field and sweet corn. Thavaprakash *et al.* (2006) investigated that foliar phosphorus applied 25 and 45 days after planting enhanced growth parameters and caused in considerably higher corn yields. Boote *et al.* (1978) indicated that foliar application of minerals such as N, P, and K support to sustain proper leaf nutrition, improves leaf N, P, and K as well as carbon balance, and encourages photosynthesis, which can lead to higher grain yields.

Haloi, (1980) described that higher rates of ammonium phosphate applied as a foliar spray to wheat not only resulted in decreased phosphorus deficiency but also directed to higher grain yields. Mosali *et al.* (2006) stated that considerably high increases in wheat grain yield are estimated with foliar phosphorus fertilisation on low P soils related to higher phosphorus fertility soils. They attained escalations in wheat grain yield when the yield levels were usually lower, maybe due to drought stress, which reduced the phosphorus uptake via contact exchange. So, one would assume the maximum response to foliar phosphorus fertilisation when moisture stress is more severe.

2.8.3. Potassium

El-Defan *et al.* (1999) reported that Soil+ foliar application of 1% and 1.5% potassium oxide increase grain yield, straw yield and 1000-grain weight. Foliar application of potassium with 1% or 5% KCl increase grain yield and number of grains per head before flowering while after flowering foliar application of potassium increase significantly protein contents and 1000-kernal weight (Abdi *et al.*, 2002). El-Abady,

(2009) conducted field experiment to study the effect of irrigation withholding i.e. without (control treatment), withholding last irrigation and last two irrigations and potassium foliar application i.e. without (control treatment), 1.5 and 3.0 % K₂O, as well as, their interaction on growth, yield and its components and grain quality of wheat cultivar Sakha 93. They also conducted a laboratory experiment to find out the germination percentage and seedling vigor tests (seed quality) of seed produced from the field experiment. The results showed that water stress during grain filling through withholding last or last two irrigations greatly reduced growth, straw and grain yields and its components as well as seed quality characters. In contrary, slight increases in grain protein content were resulted from withholding treatments in both seasons. Foliar application of potassium at the rate of 3.0 % K₂O gave the highest values of all studied wheat characters followed by 1.5 % K₂O as compared with control treatment in both seasons.

Beringer, (1980) reported that grain yield of wheat improved with increasing potassium supply. Montanee, (1989), Suwanerit and Sestopukdee (1989) reported that a solitary foliar potassium application in maize on any day between the 50% tasselling date and days later better yields and sweetness of super sweet corn. Oosterhuis *et al.* (1990) reported that cotton plant getting both soil and foliar applied potassium or foliar applied potassium only gave superior seed yields than control. El-Habbasha *et al.* (1996) stated that treating pea plants by foliar application of K resulted in an increase in the yield. Arif *et al.* (2006) reported that foliar application of nutrients to crops can balance and assurance the availability of nutrients to crops for attaining high yields.

El-Ashry *et al.* (2005) studied that foliar application of potassium to wheat plants before subjecting to drought stress reduce the adverse effects of drought on growth and increase yield/plant. Foliar application of potassium can able the plant to consume and translocate it to all other parts of the plant. Potassium treated plants significantly increase number of grains per spike, weight of grains spike⁻¹, number of spikes m⁻², straw and grain yield of wheat (El-Sabbagh *et al.*, 2002). Aziz *et al.* (2004) also reported that foliar application of potassium increase the plant height of maize. E.A.E Mesbah, (2009) reported that foliar application of 2 or 3 % potassium increase the yield of wheat plants and it also improve the water use efficiency in sandy soils. Anton and Ahmed, (2001) reported that foliar spray of potassium increased the plant height of barley.

Research work reviewed from this section, it is apparent that drought stress in the soil as well as within the plant effect numerous physiological and biochemical processes

of the plant consequently preventing plant growth, development and ultimately the yield. Water stress induced nutrient deficiency lead to decrease in growth and yield of plants due to conflicts in metabolic processes based on proteins, enzyme activity, and cellular structure. Reduction in nutrient uptake under drought stress needs to be improved in time to avoid yield losses. Several advanced approaches of fertiliser application such as foliar have shown potential in relatively alleviating the effects of drought stress-associated damages in plants. Soil application of nutrients such as nitrogen, phosphorus and potassium in this situation, can be too late and had not showed much effectiveness. Supplemental foliar-application of NPK to plants has gained reputation in current years and there are numerous studies of improved growth and yield in crops with supplemental foliar application of NPK to basal applications of soil-applied NPK.

Chapter-3

MATERIALS AND METHODS

The present study was intended to assess the response of water-stressed wheat (*Triticum aestivum* L.) to foliar application of nitrogen, phosphorus and potassium to improve the growth and yield of wheat under drought stress conditions. The study was conducted in November (spring wheat growing season in Pakistan) during the year 2011-2012.

3.1. Experimental Site and Conditions

The study was conducted in the Stress physiology Laboratory, glass house/wire house and field conditions at Department of Crop Physiology, University of Agriculture Faisalabad, with the collaboration of Stress Physiology Laboratory, Salinity and Environmental Division, Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan. A series of laboratory, glass house/wire house and field experiments were conducted for this study. The laboratory experiments were conducted in petri plates and plastic cups containing 400 g of sterilized sand whereas the glass house/wire house experiments were conducted in plastic pots (15 × 10 cm) containing 2 kg of sterilized sand. Laboratory and glass house experiments were laid out in completely randomized design (CRD). The wire house experiment was conducted at Department of Crop Physiology wire house laid out in a randomized complete block design (RCBD) factorial in split plot arrangement with three repeats. The field experiments were also laid out in randomized complete block design (RCBD) factorial in split plot arrangement with three repeats in sandy clay loam soil at research area, Department of Crop Physiology, University of Agriculture, Faisalabad. The soil texture was determined with the hygrometer method (Dewis and Freitas, 1970). The physiochemical characteristics (Electrical conductivity, pH and ion contents) of the soil used for this study were determined according to methods described by Jackson, (1962) and are present in Table 3.1. The weather in respect of minimum and maximum (°C), relative humidity (%) and rainfall (mm) of the experimental site (both wire house and field experiment) for the year 2011-2012 and 2012-13 is given in the Figure 3.1 and 3.2 respectively.

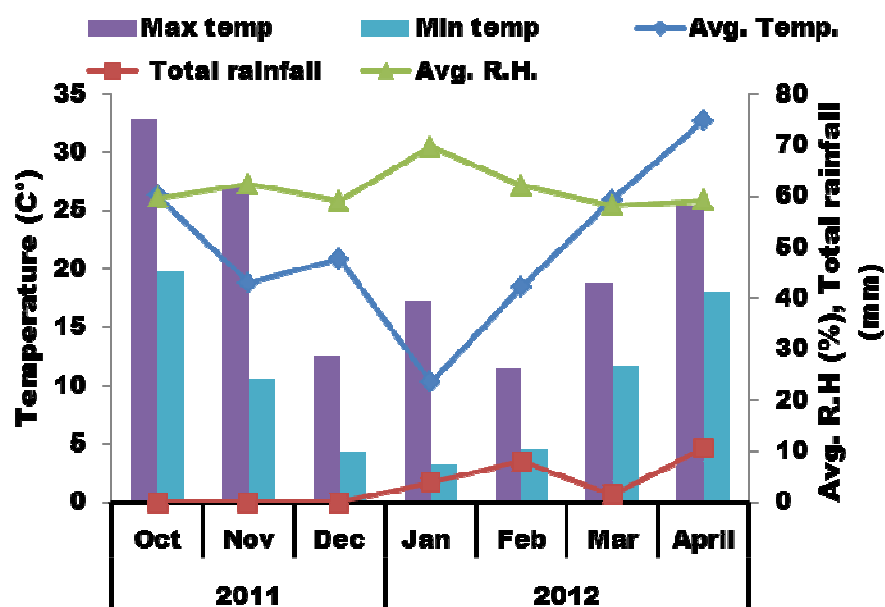


Fig. 3.1: Meteorological data of the experimental site for Field experiment for the growing season 2011-12

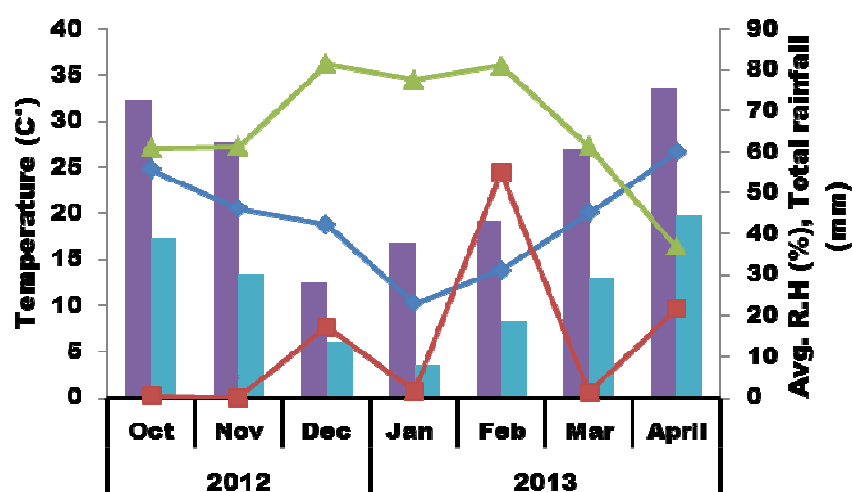


Fig. 3.2: Meteorological data of the experimental site for Field experiment for the growing season 2012-13.

Table.3.1: Physiochemical characteristics of the soil used for field experiments.

Soil Characteristics	Values
Physical	
Soil texture	Clay loam
Chemical	
Saturation percentage (%)	37.4
Soil pH _s	7.5-7.8
Organic matter (%)	0.4-0.6
EC _e (dSm ⁻¹)	0.72-0.92
HCO ₃ (meq L ⁻¹)	3.5-4.0
Ca+Mg (meq L ⁻¹)	3.75-5.76
CO ₃ (meq L ⁻¹)	Nil
NO ₃ -N (mg kg ⁻¹)	10.7-14.7
Available P (mg kg ⁻¹)	8.2-10.8
Available K (mg kg ⁻¹)	108-200

3.2. Wheat Germplasm Collection

The seeds of ten local wheat genotypes viz. Lasani 2008, Shafaq 06, Ufaq 06, Chakwal-86, Farid 06, Miraj-06, Manthar 03, Bhakkar 02, FSD-08 and V₀ 4178 were used for the screening of these genotypes for drought tolerance. The seeds were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad.

3.3. Laboratory Experiments

3.3.1. Germination

The experiments were carried out at Stress Physiology Laboratory, University of Agriculture, Faisalabad. During the first experiment, polyethylene glycol (PEG) with the molecular weight of 6000 was used as a drought stimulator and five stress levels of zero (control), -0.2, -0.4, -0.6 and -0.8 MPa were developed by dissolving 6.65, 13.30, 20 and 26.6 g of PEG separately in 100 ml of distilled water. The concentrations of the solution were confirmed by Viesper Osmometer (Model 5520) at 25 °C according to the method of Michel and Kaufmann, (1973).

Local wheat genotypes viz. Lasani 2008, Shafaq 06, Ufaq 06, Chakwal-86, Farid 06, Miraj-06, Manthar 03, Bhakkar 02, FSD-08 and V₀ 4178 were evaluated for drought tolerance. Randomly selected 20 seeds of each cultivar were sterilized for five seconds with 5% sodium hypochlorite solution, washed them with distilled water and then air dried. Seeds were placed in covered sterilized petri dishes having 9 cm diameter and containing filter paper moistened with 10 ml of PEG-6000.

The data for germination percentage, germination index (AOSA, 1983), promptness index (Ashraf *et al.*, 2006) and germination stress index (Fernandez, 1992) were recorded till eight days. At the end of 8th day, randomly selected five seedlings from each petri plate were harvested for the measurement of plant fresh and dry weight to calculate the vigor index. Germination data were recorded on daily basis. Seeds were considered to be germinated when gained approximately 2 mm of root length and used for germination percentage calculations (Afzal *et al.*, 2004). Complete germination of seeds was considered when no further germination occurred in two successive days.

Recorded data was used for calculation of promptness index (PI) and germination stress tolerance index (G.S.I) according to the formulae given by Ashraf *et al.* (2006).

$$P.I = nd_2 (1.00) + nd_4 (0.75) + nd_6 (0.5) + nd_8 (0.25)$$

Where n is the number of seeds germinated on day d

$$G.S.I. = (P.I \text{ of stressed seeds} / P.I \text{ control seeds}) \times 100$$

The germination index was calculated as:

$$G.I = (\text{number of seeds germinated/days of first count}) + \dots + (\text{number of seeds germinated/days of final count})$$

(AOSA, 1983)

3.3.2. Seedling Growth

The second experiment was conducted under laboratory conditions in pots. The seeds of ten wheat genotypes/line (Lasani 2008, Shafaq 06, Ufaq 06, Chakwal-86, Farid 06, Miraj-06, Manthar 03, Bhakkar 02, FSD-08 and V₀ 4178) were grown for 28 days in plastic pots having sand as a growth medium. All the pots were watered daily at 100 % field capacity for 8 days. After the completion of seed germination the pots were divided into two sets. One set of pots was watered daily at 100% field capacity while in other set the irrigation was stopped to impose water stress. The data on Root length stress index (RLSI), dry matter stress index (DMSI) and plant height stress index (PHSI) were recorded after four weeks of seedling growth by using the following formulae given by Ashraf *et al.* (2006).

$$PHSI = [\text{Plant height of stressed plant} / \text{Plant height of control plant}] \times 100$$

$$DMSI = [\text{Dry matter of stressed plant} / \text{Dry matter of control plant}] \times 100$$

$$RLSI = [\text{Root length of stressed plant} / \text{Root length of control plant}] \times 100$$

Significant differences among cultivar means were determined by analysis of variance according to Duncan's Multiple Range Test ($P < 0.05$) in an experiment with factorial structure of treatments evaluated in a completely randomized design with three repeats for each variable.

The data so collected was analyzed statistically using analysis of variance technique and the STATISTICA Computer Program was used for this purpose.

3.4. Glass House Experiment

A pot experiment was conducted in glass house for optimization of foliar applied nutrients helpful in improving drought tolerance in wheat plants subjected to water stress at seedling stage. One drought tolerant (Bhakkar-02) and sensitive genotype (Shafaq-06) selected from laboratory experiments were used for this experiment. Randomly selected 30 seeds were taken from 100 g of seeds of each cultivar and were sterilized for five minutes with 5% sodium hypochlorite solution and washed three times with distilled water. Initially, each replication consisted of ten seeds of each cultivar sown in plastic pots (15×10 cm) containing 2 kg of sterilized sand as growth medium but only five plants were kept after thinning in each replication.

The treatments were control, water spray, N (alone) 1.5% Zhang *et al.* (2009), P (alone) 2% dos Santos *et al.* (2004), K (alone) 3% El-Abady *et al.* (2009), N and P (50%-50%), N and K (50%-50%), P and K (50%-50%) and N, P, K (33%-33%-33%). In this experiment, all the pots were watered daily at 100 % field capacity for 8 days. After the completion of seed germination the pots were divided into two sets. One set of pots was watered daily at 100% field capacity while in other set the irrigation was stopped to impose water stress. The plants were foliar sprayed of nitrogen, phosphorus, potassium and with their combinations after three days of imposition of stress and were harvested after four weeks. The experiment was repeated three times to record data regarding physiological indices and total biomass of plants. Completely randomized design (CRD) with three replications was used for these experiments. The data on Root length stress index (RLSI), dry matter stress index (DMSI) and plant height stress index (PHSI) were recorded after four weeks of seedling growth by using the following formulae given by Ashraf *et al.* (2006).

$$\text{PHSI} = [\text{Plant height of stressed plant} / \text{Plant height of control plant}] \times 100$$

$$\text{DMSI} = [\text{Dry matter of stressed plant} / \text{Dry matter of control plant}] \times 100$$

$$\text{RLSI} = [\text{Root length of stressed plant} / \text{Root length of control plant}] \times 100$$

3.4.1. Physiological Parameters

3.4.1.1 Leaf Water Potential

Fully expanded youngest leaf of plants from each treatment was used to determine the leaf water potential. The measurements were made from 8.00 to 10.00 a.m. with Scholander type pressure chamber.

3.4.1.2. Leaf Osmotic Potential

The same leaf, as used for water potential, was frozen at -20°C for osmotic potential determinations. The frozen leaf material was thawed and cell sap was extracted while crushing the leaves with a glass rod and then sap was sucked with a disposable syringe. The sap so extracted was directly used for the determination of osmotic potential using an osmometer (Wescor 5500).

3.4.1.3. Turgor Potential

Turgor potential was calculated as the difference between osmotic potential (ψ_s) and water potential (ψ_w) values.

$$(\psi_p) = (\psi_w) - (\psi_s)$$

3.4.2. Gas Exchange Characteristics

A fully expanded youngest leaf of each plant (the third leaf from top) was used to measure the instantaneous net CO₂ assimilation rate (A), transpiration (E) and stomatal conductance (gs) by using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). These measurements were taken from 9.00 to 11.00 a.m. with the following adjustments: molar flow of air per unit leaf area 403.3 mmol m⁻²S⁻¹, atmospheric pressure 99.9 kPa, water vapor pressure into chamber ranged from 6.0 to 8.9 m bar, PAR at leaf surface was maximum up to 1711 mol m⁻² s⁻¹, temperature of leaf ranged from 28.4 to 32.4 °C, ambient temperature ranged from 22.4 to 27.9°C and ambient CO₂ concentration was 352 mol mol⁻¹.

Significant differences among cultivar means were determined by analysis of variance according to Duncan's Multiple Range Test (P < 0.05) in an experiment with factorial structure of treatments evaluated in a completely randomized design with three repeats for each variable.

The data so collected was analyzed statistically using analysis of variance technique and the STATISTICA Computer Program was used for this purpose.

3.5. Wire House Experiment

A pot experiment was conducted in rain-out shelter to optimize the time (vegetative or reproductive growth stage) of best combination that is helpful in improving drought tolerance in water stressed wheat plants. In this experiment, two wheat genotypes i.e. one drought tolerant (Bhakkar-02) and one drought sensitive (Shafaq-06) as used in glass house experiments were grown under normal (100% field capacity) and water stress (60% field capacity) levels.

3.5.1. Seed Sowing

For wire and glass house experiments sand was initially sun dried, ground, sieved and mixed well in order to avoid any plant residues. For wire house experiment 2 kg sand was filled carefully in each pot. Five seeds were sown in each pot and then watered with distilled water. In the beginning all pots were kept at field capacity level for obtaining good germination and emergence. Later on the water was applied according to the water stress level specified for that experiment. Before imposing water stress the plants were thinned out and three healthy plants were kept in each pot. Basal nutrients in solution form were applied to each pot at the following rates (ml kg^{-1} sand): NH_4NO_3 , 1.67; KH_2PO_4 , 1.67; K_2SO_4 , 3.33; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.67; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 3.33; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 1.67; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.67; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 1.67; H_3BO_3 , 1.67; $\text{CaSO}_4 \cdot 7\text{H}_2\text{O}$, 1.67; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 1.67. Nitrogen in the form of NH_4NO_3 , 1.67 ml kg^{-1} was applied after every 2 weeks.

3.5.2 Development and Maintenance of Water Stress Levels.

Water stress was imposed after germination by applying less amount of water in water stressed pots. For each treatment pots were weighed daily at about 9:00 am, calculated the amount of water consumed in evapotranspiration and watered until the pot weight reached to pre-determined weight. In wire house experiment pots were watered at 100% field capacity and 60% field capacity.

Plants were grown up to maturity and data regarding various agronomic, physiological, biochemical parameters and nutrients analysis was recorded using standard recommended methods.

3.5.3: Physiological Parameters

3.5.3.1. Leaf Water potential

The third leaf from top (fully expanded youngest leaf) of plants from each treatment was used to determine the leaf water potential. The measurements were made from 8.00 to 10.00 a.m. with Scholander type pressure chamber.

3.5.3.2. Leaf Osmotic Potential

The same leaf, as used for water potential, was frozen at -20°C for osmotic potential determinations. The frozen leaf material was thawed and cell sap was extracted while crushing the leaves with a glass rod and then sap was sucked with a disposable syringe. The sap so extracted was directly used for the determination of osmotic potential using an osmometer (Wescor 5500).

3.5.3.3. Turgor Potential

Turgor potential was calculated as the difference between osmotic potential (ψ_s) and water potential (ψ_w) values.

$$(\psi_p) = (\psi_w) - (\psi_s)$$

3.5.3.4. Relative Water Contents (RWC)

Flag leaf samples were taken from three plants of each treatment. Fresh weight (FW) of each sample was taken on digital electrical balance and dipped in test tube containing distilled water for 24 hours. Then it was wiped with the tissue paper and turgid weight (TW) was taken. The samples were dried at 65°C for 72 hrs and dry weight (DW) of each sample was taken. For each treatment, RWC was calculated by using the formula (Karrou and Maranville, 1995) given below:

$$RWC = [(FW-DW) / (TW-DW)] \times 100$$

Where FW= fresh weight, DW= dry weight and TW= turgid weight

3.5.3.5. Gas Exchange Characteristics

A fully expanded youngest leaf of each plant at vegetative stage and flag leaf at reproductive stage was used to measure the instantaneous net CO₂ assimilation rate (A), transpiration (E) and stomatal conductance (gs) by using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). These measurements were taken from 9.00 to 11.00 a.m. with the following adjustments: molar flow of air per unit leaf area 403.3 mmol m⁻²S⁻¹, atmospheric pressure 99.9 kPa, water vapor pressure into chamber ranged from 6.0 to 8.9 m bar, PAR at leaf surface was maximum up to 1711 mol m⁻² s⁻¹, temperature of leaf ranged from 28.4 to 32.4 °C, ambient temperature ranged from 22.4 to 27.9°C and ambient CO₂ concentration was 352 mol mol⁻¹.

3.5.4. Biochemical parameters

3.5.4.1: Chlorophyll Contents

Chlorophyll contents were calculated by using the method of Arnon, (1949) and Davies, (1976). Fresh leaves of (0.5 g) were chopped into segments of 0.5 cm and extracted with 5 mL acetone (80%) at 10°C overnight. Centrifuge the material at 14000 rpm for 5 min. and measured the absorbance of the supernatant at 645, 652 and 663 nm on spectrophotometer. Calculated a, b and total chlorophyll.

$$\text{Chl a} = [12.7 (\text{OD } 663) - 2.69 (\text{OD } 645)] \times V/1000 \times W$$

$$\text{Chl b} = [22.9 (\text{OD } 645) - 4.68 (\text{OD } 663)] \times V/1000 \times W$$

$$\text{Total Chl} = [20.2 (\text{OD } 645) + 8.02 (\text{OD } 663)] \times V/100 \times W$$

$$\text{Carotenoids (g.mL}^{-1}\text{)} = A^{\text{car}}/E_{\text{max}}^{100}$$

Where V is the volume of sample extract and W is the weight of the sample and

$$A_{\text{car}} = (\text{OD}480) + 0.114 (\text{OD}663) - 0.638 (\text{OD}645); E_{\text{max}}^{100} \text{ cm} = 2500$$

Where V is the volume of sample extract and W is the weight of the sample.

3.5.4.2. Total Soluble Protein

Total soluble proteins were determined using the method of Lowry *et al.* (1951).

Reagents

Phosphate buffer (0.2 M): Following chemicals were used to prepare the phosphate buffer.

1. One-molar solution of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (156.01 g L^{-1}) was prepared as the stock.
2. One-molar solution of Di-sodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 177.99 g L^{-1}) was prepared as the stock.

Copper Reagents

Solution A

Na_2CO_3 = 2.0 g

NaOH = 0.2 g

Sodium potassium tartarate = 1.0 g

All the three chemicals were dissolved in distilled water and the volume was made to 100 mL.

Solution B

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ solution: 0.5g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was dissolved in 100 mL distilled water

Solution C

Fifty mL of solution A and 1.0 mL of solution B were mixed to prepare alkaline solution. This solution was always prepared fresh.

Folin Phenol Reagent

One hundred g of sodium tungstate and 25 g of sodium molybdate were dissolved in 700 mL of distilled water. Fifty mL of 85% orthophosphoric acid and 100 mL of HCl were added and the mixture was refluxed for 10 h. Then 150 g of lithium sulfate was added along with 50 mL of distilled water. A few drops of Br_2 were also added.

The mixture was boiled without condenser for 15 min to remove extra Br_2 . The mixture was then cooled and diluted to 1000 mL.

Standard Bovine Serum Albumin (BSA) solution ($1 \mu\text{g mL}^{-1}$).

Ten mg of Bovine serum albumin (BSA) was dissolved in 10.0 mL of distilled water.

Extraction

Fresh leaf material (0.5 g) was chopped in 10 mL of phosphate buffer (0.2 M) of pH 7.0 and was ground. The ground leaf material was centrifuged at $5000 \times g$ for 5 min. The supernatant was used for protein determination.

Procedure

One mL of the leaf extract from each treatment was taken in a test tube. The blank contained 1 mL of phosphate buffer (pH 7.0). One mL of solution C was added to each test tube. The reagents in the test tube were thoroughly mixed and allowed to stand for 10 min at room temperature. Then 0.5 mL of Folin-Phenol reagent (1:1 diluted) was added, mixed well and incubated for 30 min. at room temperature. The optical density (OD) was read at 620 nm on a spectrophotometer (Hitachi, 220, Japan).

3.5.4.3. Total Free Amino Acids

Total free amino acids were determined according to Hamilton and Van Slyke (1973). Fresh plant leaves (0.5 g) were chopped and extracted with phosphate buffer (0.2 M) having pH 7.0. Took 1 mL of the extract in 25 mL test tube, added 1 mL of pyridine (10 %) and 1mL of ninhydrin (2 %) solution in each test tube. Ninhydrin solution was prepared by dissolving 2 g ninhydrine in 100 mL distilled water. The test tubes with sample mixture, heated in boiling water bath for about 30 min. Volume of each test tube was made up to 50 mL with distilled water. Read the optical density of the coloured solution at 570 nm using spectrophotometer. Developed a standard curve with Leucine and calculated free amino acids using the formulae given below:

$$\text{Total amino acids} = \frac{\text{Graph reading of sample} \times \text{Volume of sample} \times \text{Dilution factor}}{\text{Weight of fresh tissue} \times 1000}$$

(μg g⁻¹ fresh wt)

3.5.4.4. Total Soluble Sugars

Total soluble sugars were determined according to the method of Yemm and Willis, (1954).

Extraction

Dried plant material was ground well in a micromill and the material was sieved through 1 mm sieve of micromill. Plant material (0.1 g) was extracted in 80% ethanol solution. The extract was incubated for 6 h at 60 °C. This extract was used for the estimation of total soluble sugars.

Reagents

Anthrone reagent was prepared by dissolving 150 mg of anthrone in 72% H₂SO₄ solution. This reagent was freshly prepared whenever needed.

Procedure

Plant extract was taken in 25 mL test tubes and 6 mL anthrone reagent was added to each tube, heated in boiling water bath for 10 min. The test tubes were ice-cooled for 10 min. and incubated for 20 min. at room temperature (25°C). Optical density was read at 625 nm on a spectrophotometer (Hitachi, 220, Japan). The concentration of soluble sugars was calculated from the standard curve developed by using the above method.

3.5.4.5. Proline Determination

The proline was determined according to the Bates *et al.* (1973) method. Fresh leaf material of 0.5 g was ground and dissolved in 10 mL of 3% sulfo-salicylic acid. The sample material was filtered by using Whatman No. 2 filter paper. Two mL of the filtrate was taken in a test tube and reacted with 2 mL acid ninhydrin solution. Acid ninhydrin solution was prepared by dissolving 1.25 g ninhydrine in 30 mL of glacial acetic acid and 20 mL of 6 M orthophosphoric acid. Two mL of glacial acetic acid was added in the test tube and kept for 1 h at 100 °C. After terminating the reaction in an ice bath, the reaction mixture was extracted with 10 mL toluene. Continuous air stream was passed vigorously for 1-2 minutes in the reaction mixture. The chromophore containing toluene was aspirated from the aqueous phase, warmed at room temperature and the absorbance was noted at 520 nm on spectrophotometer. Toluene was used as a blank. The proline concentration was calculated by using a standard curve and calculated on fresh weight basis as follows:-

mmole proline g⁻¹ fresh weight = (g proline mL⁻¹ x mL of toluene/115.5)/ (wt of sample/5)

3.5.5. Antioxidant Enzymes

The activities of POX, CAT, and ascorbate peroxidase (APX) were determined spectrophotometrically. Leaves were homogenized in a medium composed of 50 mM phosphate buffer with 7.0 pH and 1 mM dithiothreitol (DTT) as described by Dixit *et al.* (2001).

3.5.5.1. Catalase (CAT)

Catalase activity was assayed by measuring the conversion rate of hydrogen peroxide to water and oxygen molecules, following the method described by Chance and Maehly (1955). The activity was assayed in 3 mL reaction solution comprising 50 mM phosphate buffer with 7.0 pH, 5.9 mM of H₂O₂ and 0.1 mL enzyme extract. The catalase activity was determined by decline in absorbance at 240 nm after every 20 sec due to consumption of H₂O₂. Absorbance change of 0.01 units min⁻¹ was defined as one unit catalase activity.

3.5.5.2. Peroxidase (POX)

The activity of POD was determined by measuring peroxidation of hydrogen peroxide with guaiacol as an electron donor (Chance and Maehly, 1955). The reaction solution for POD consists of 50 mM phosphate buffer with pH 5, 20 mM of guaiacol, 40 mM of H₂O₂ and 0.1 mL enzyme extract. The increase in the absorbance due to the formation of tetraguaiacol at 470 nm was assayed after every 20 sec. One unit of the enzyme was considered as the amount of the enzyme that was responsible for the increase in OD value of 0.01 in 1 min. The enzyme activity was determined and expressed as units min⁻¹ g⁻¹ fresh weight basis.

3.5.5.3. Ascorbate Peroxidase Activity

Ascorbate peroxidase (APX) activity was measured by monitoring the decrease in absorbance of ascorbic acid at 290 nm (extinction coefficient 2.8 mM cm⁻¹) in a 1 mL reaction mixture containing 50 mM phosphate buffer (pH 7.6), 0.1 mM Na-EDTA, 12 mM H₂O₂, 0.25 mM ascorbic acid and the sample extract as described by Cakmak, (1994).

3.5.6. Enzymes

3.5.6.1. Nitrate Reductase Activity

Nitrate reductase was estimated according to Sym, (1984) method. Fresh leaf samples (0.5 g) was cut into 0.5 cm pieces in a test tube containing 4.5 mL of 0.02 M phosphate buffer (pH 7.0), prepared by dissolving sodium dihydrogen phosphate and disodium hydrogen phosphate containing 0.1 % triton X-100. Prepared 0.02 M KNO₃ solution in phosphate buffer of pH 7.0 and added 0.5 mL in the test tube. Incubated these test tubes at 32 °C for 1 hour in dark. Took 1 mL of reaction medium and reacted with 0.5 mL of sulphanilamide (1 g of sulphanilamide in 100 mL of 2N HCl). After shaking well 0.5 mL of N (1-naphthyl) ethylene diamine dihydrochloride (0.02 g of N (1-Naphthyl)-ethylene diamine dihydrochloride in 100 ml of distilled water) was added immediately. Pink diazo colour complex with NO₂ was appeared. After 20 minutes the colour was diluted with 5mL distilled water and centrifuged for 5 minutes at 2000 rpm to remove turbidity. Read the absorbance at 542 nm on spectrophotometer against a set of standards developed with NaNO₂. Blank was consisting 1 mL of incubated buffer with all chemicals. The activity is expressed as presence of $\mu\text{mol NO}_2 \text{ g}^{-1} \text{ fresh weight h}^{-1}$.

3.5.6.2. Nitrite Reductase Activity

Nitrite reductase was estimated according to Sym, (1984) method. Fresh leaves material (0.5 g) was chopped in 4.5 mL of 0.2 M phosphate buffer (pH 5.0) and 0.5mL of NaNO₂ (0.02 M) was added in mixture sample. Prepared 0.02 M sodium nitrite solution in phosphate buffer of pH 5.0. Evacuated the tubes for 2 min with a pump and were wrapped in aluminum foil. The test tubes were incubated at 30 °C in a water bath with gentle shaking for 30 min. Transferred the tubes in boiling water to terminate the reaction and then cooled at room temperature. Took 1mL of the extract and reacted with 0.5 mL of 1% sulphanilamide, prepared by dissolving 1 g of sulphanilamide in 100 mL of 2N HCl and 0.5 mL of 0.02% aqueous solution of N (1-Naphthyl)-ethylene diamine dihydrochloride, prepared by dissolving 0.02 g of N(1-Naphthyl)-ethylene diamine dihydrochloride in 100 mL of distilled water. Allowed the mixture for 20 minutes to develop colour. The volume of the reactants was made up to 10 ml with distilled water and the optical density read at 540nm on a spectrophotometer. Developed the standard curve with NaNO₂. Follow all above procedure with substrate NO₂ and blank containing no NaNO₂. The activity is expressed as presence of $\mu\text{mol NO}_2 \text{ g}^{-1} \text{ fresh weight h}^{-1}$.

3.5.7. Nutrients Analysis

3.5.7.1. Estimation of Nitrogen (N)

Nitrogen was estimated by micro-Kjeldhal method (Bremmer, 1965). Following reagents were used for N determination.

1. Boric acid solution (3%)
2. Sulphuric acid standard (0.01 N)
3. Mixed indicator of bromocresol green and methyl red

Procedure

The digested material (5 mL) was taken in Kjeldhal tubes. The tubes were placed on the Kjeldhal ammonia distillation unit and 5 mL of 40% NaOH were added to each tube. Boric acid solution (5 mL) was taken in a conical flask with few drops of mixed indicator. When the distillate was approximately 40 mL, the distillation was stopped. The distillate was cooled for few minutes and titrated it against 0.01 N standard H₂SO₄ till the solution turned pink. A blank was run for the complete procedure.

N was estimated by the following formula.

$$\text{N \%age} = \frac{(V_2 - V_1) \times N \times 0.014 \times 100}{W}$$

Where

V₂ = Volume of standard H₂SO₄ required to titrate the sample solution.

V₁ = Volume of standard H₂SO₄ required to titrate the blank solution.

N = Normality of H₂SO₄

W = Weight of the sample

3.5.7.2. Estimation of Phosphorus

Phosphorus (P) was determined by a spectrophotometer (Jackson, 1962). The extracted material (2 mL) was dissolved in 2 mL of Barton's reagent and total volume was made 50 mL. These samples were kept for half an hour before analyzing P.

The values of P were calculated by using standard curve. The Barton's reagent was prepared as described below:

Barton's Reagent

Solution A:

25 g of ammonium molybdate was dissolved in 400 mL of distilled water.

Solution B:

Ammonium metavanadate (1.25 g) was dissolved in 300 mL of boiling water, cooled, and 250 mL of conc. HNO₃ were added. The solution was again cooled at room temperature.

The solution A and B were mixed and the volume was made to 1 L. It was stored at room temperature.

3.5.7.3. Estimation of K

Digestion

Dried ground material (0.5 g) was taken in digestion tubes and 5 mL of concentrated H₂SO₄ were added to each tube (Wolf, 1982). All the tubes were incubated overnight at room temperature. Then 0.5 mL of H₂O₂ (35%) poured down the sides of the digestion tube, ported the tubes in a digestion block and heated at 350 °C until fumes were produced. They were continued to heat for another 30 min. The digestion tubes were removed from the block and cooled. 0.5 mL of H₂O₂ was slowly added and the tubes were placed back into the digestion block. The above step was repeated till the cooled digested material was colorless. The volume of the extract was maintained up to 50 mL in volumetric flasks. The extract was filtered and used for determining K, P and N.

Estimation of cations (K⁺)

Potassium (K⁺) was determined using flame photometer (Jenway PFP 7).

3.6. Field Experiments

Field experiments were conducted over two years at research area of Department of Crop Physiology, University of Agriculture, Faisalabad known best combination of Nitrogen, Phosphorus, and Potassium using one drought tolerant and one drought sensitive wheat variety. Best combination of Nitrogen, Phosphorus, and Potassium selected from glass house experiments were applied at two water stress levels i.e at tillering and anthesis. The stress was imposed by withholding the irrigation. During 2011-12, the no stress treatment received 397 mm water (375 mm irrigation + 22 mm rainfall) during the whole wheat growing season, whereas water- stressed plants received 322 mm water (300 mm irrigation + 22 mm rainfall). Similarly in the next year (2012-13), the normal plants received 473 mm water (375 mm irrigation + 98 mm rainfall), whereas the plants exposed to drought stress received 398 mm water (300 mm irrigation + 98 mm) during the whole crop growth period. The experiment was laid out in RCBD factorial in split plot arrangement. Plants were allowed to grow up to maturity. Recommended dose of N, P and K as urea (110 kg N ha^{-1}), diammonium phosphate ($70 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$) and potassium sulphate ($50 \text{ kg SOP ha}^{-1}$), respectively. All P and K were applied at the time of sowing but N was applied in three split doses. Data regarding yield and yield components were recorded. Same experiment was conducted in the next growing season to confirm the above results.

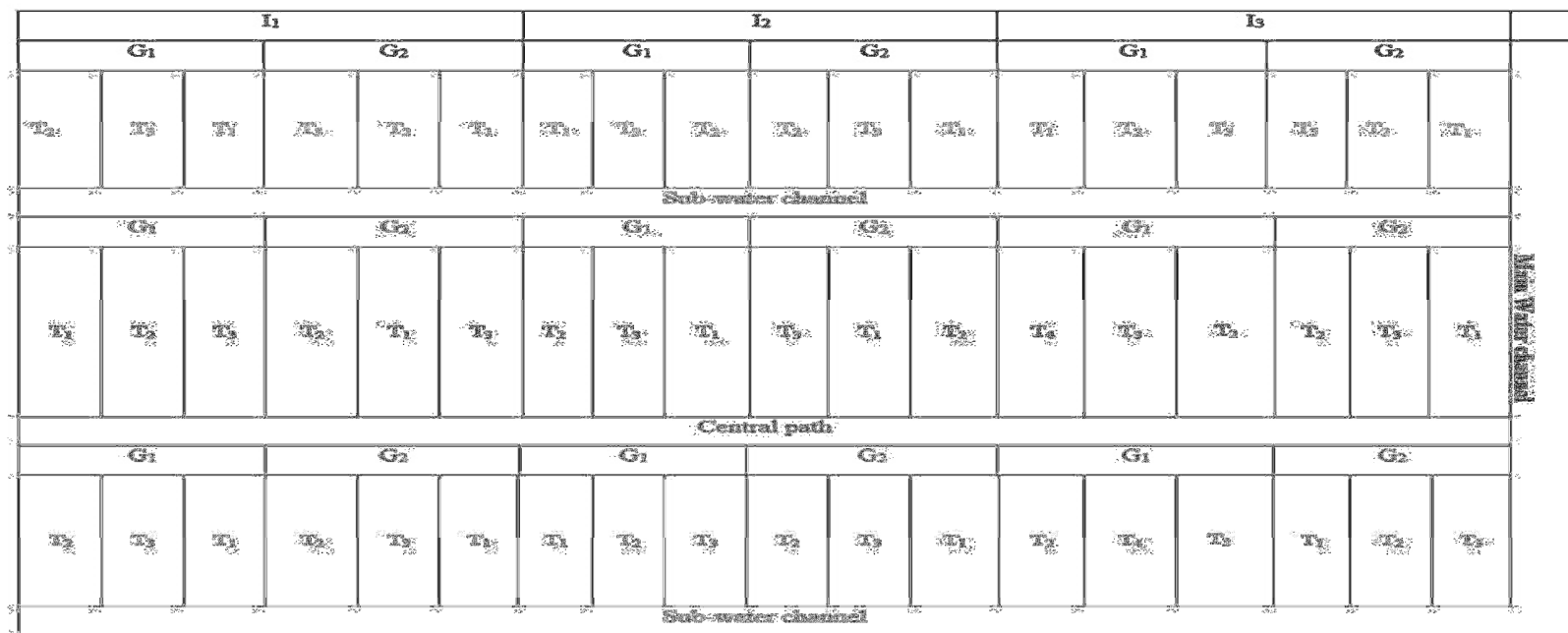
3.6.1. Land Preparation

The land was deep plowed to dry the soil. Field was irrigated 8 days before planting wheat and was plowed at a time when the field is neither so wet that structure was destroyed; not so dry that big clods were produced. A wooden plank was dragged over the soil to break the clods and make fine, smooth seedbed.

3.6.2. Time, Rate and Method of Planting

The wheat sowing was done on 17th November. Wheat was seeded at the rate of 110 kg ha^{-1} by pora method. Weeds were removed through hand weeding. Wheat was harvested on 10th of April. The following yield and yield components were recorded:

Layout Plan for Field Experiment



Treatments:

T₁: No spray

T₂: NPK spray at tillering

T₃: NPK spray at anthesis

I₁: Five Irrigations

I₂: Three Irrigations

I₃: Three irrigations

Genotypes:

G₁: Shafaq-06 (Drought sensitive)

G₂: Bhakkar-02 (Drought tolerant)

Plot Size:

Net plot size = 1.9x6m; RxR = 9 inches; No. of rows in each plot = 6

Number of Replications = 3; Total area = 342 m²

3.6.3. Yield and Yield Components

3.6.3.1. Number of Tillers m⁻²

A steel quadrant of one meter square was used to record number of tillers m⁻². Total number of tillers of randomly selected quadrant in each plot was counted at the final harvest and then average number of tillers of each plot was recorded

3.6.3.2. Number of Fertile Tillers m⁻²

Productive tillers of randomly selected quadrates in plot were counted by subtracting the non-productive tillers from total number of tillers.

3.6.3.3. Plant Height

To record plant height, five plants of each plot were measured separately and then average plant height was calculated.

3.6.3.4. Spike Length

To record spike length, five spikes of each plant were measured separately and then average spike length was calculated.

3.6.3.5. Number of Spikelets per Spike

Five spikes were removed from randomly selected plant in each plot. Numbers of spikelets in each spike were counted and average was calculated.

3.6.3.6. Number of Grains per Spike

Five spikes taken randomly from each pot were threshed manually, their grains were counted and average was worked out.

3.6.3.7. 1000-Grains Weight

The produce of plants was counted; their weight was recorded with the help of an electric balance to get 1000-grain weight.

3.6.3.8. Biological Yield

Biological yield per meter square was calculated by weighing plants on an electric balance.

3.6.3.9. Grains Yield

The grain yield per meter square was calculated after threshing plants manually and weighing grains on an electric balance.

3.6.3.10. Harvest Index (%)

It was recorded for each pot by using the formula:

$$HI = \frac{\text{Economic yield (grain yield)}}{\text{Biological yield (grain + straw)}} \times 100$$

3.6.3.11. Statistical Analysis

Data so collected in different experiments of this study were analyzed statistically using analysis of variance technique and the STATISTIX Computer Program was used for this purpose. The Least Significant Difference test at 5% probability level was used to assess the differences among significant means (Steel *et al.*, 1997).

Chapter-4

RESULTS

4.1. Laboratory Experiment-1

4.1.1. Germination

The analysis of variance showed a highly significant ($P \leq 0.001$) interaction between genotypes and PEG-6000 induced stress levels for GP, GI, PI, and GSI. In all tested genotypes, the non-stress control treatment (0 MPa) recorded the maximum GP, GI, PI, and GSI which consistently decreased in response to increasing osmotic stress. (Figs.4.1-4.4). Drought tolerant Bhakkar-02 recorded the maximum GP (100%), GI (12.6), PI (30.3), and GSI (97%) in control treatment. Drought sensitive, Shafaq-06 recorded the minimum GP (40%), GI (5.3), PI (13.2), and GSI (50%) in the treatment where water stress was imposed at (-0.8 MPa). The GI of Bhakkar-02 (12.6) was statistically at par with Lasani-08 under non-stress conditions. The Bhakkar-02 and Lasani-08 were also statistically at par in treatment where water stress was imposed at (-0.2 MPa). The maximum GSI (Table 4.1) of Bhakkar-02 was statistically at par with Fareed-06 and Lasani-08 in the treatment where water stress was imposed at (-0.2 MPa).

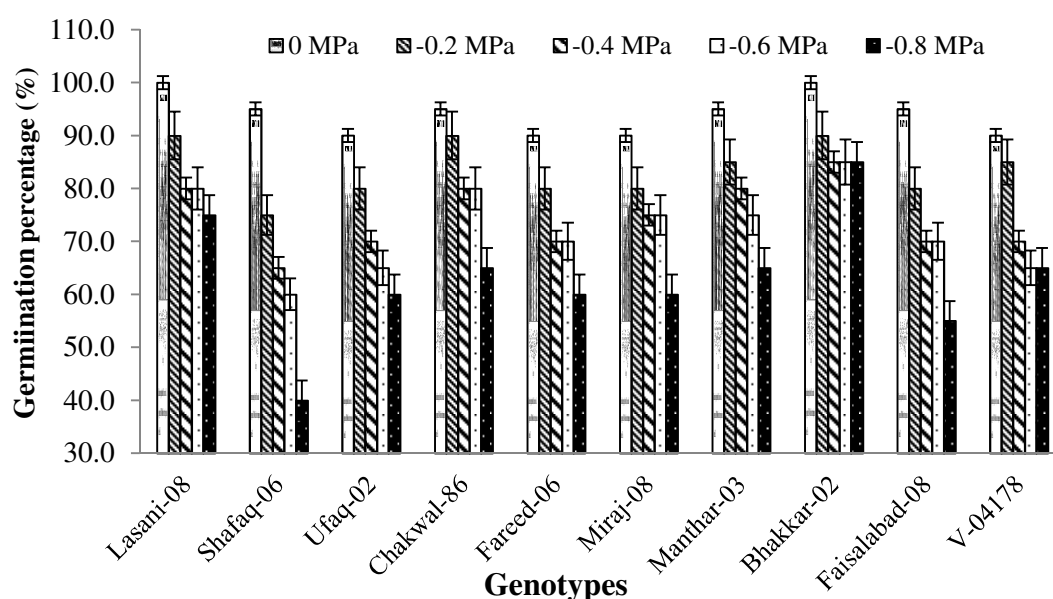


Fig. 4.1: Germination percentage of ten wheat (*Triticum aestivum* L.) genotypes under PEG (6000) induced water stress regimes (mean values $\pm$ S.E).

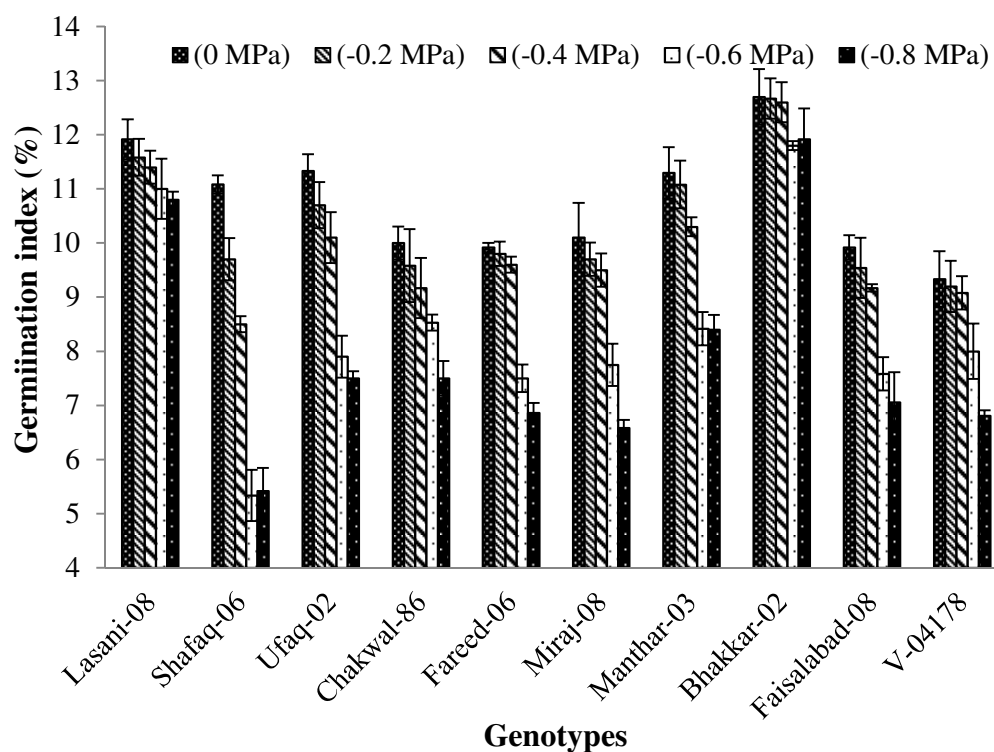


Fig. 4.2: Germination index (GI) of ten wheat (*Triticum aestivum* L.) genotypes under PEG (6000) induced water stress regimes (mean values $\pm$ S.E.).

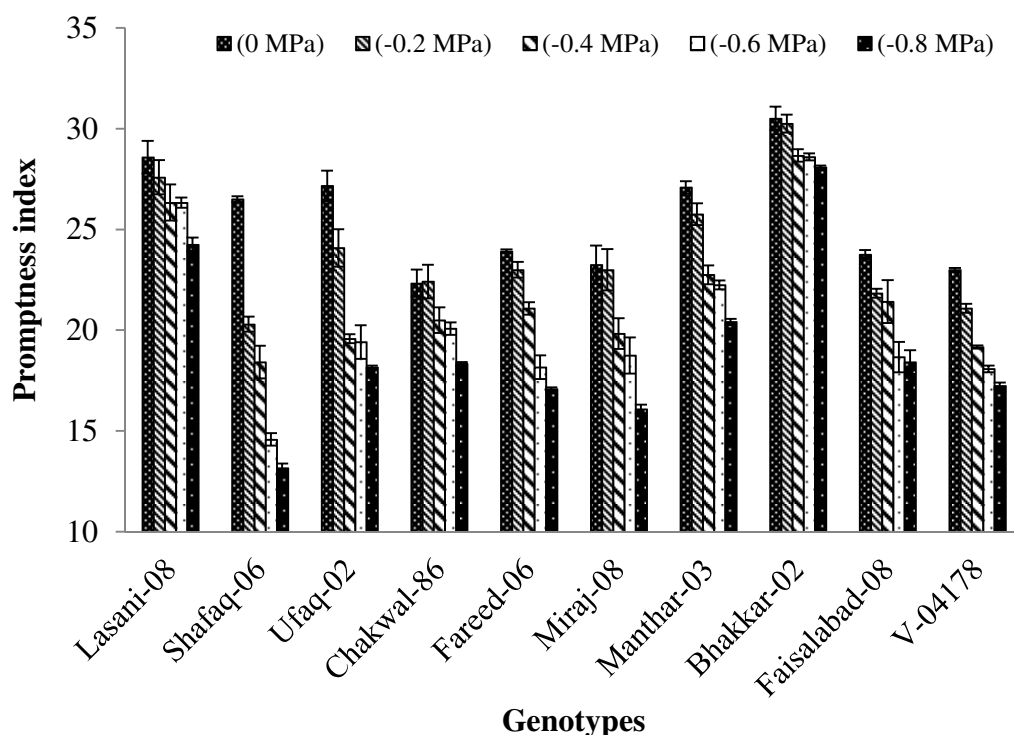


Fig. 4.3: Promptness index (PI) of ten wheat (*Triticum aestivum* L.) genotypes under PEG (6000) induced water stress regimes (mean values $\pm$ S.E.).

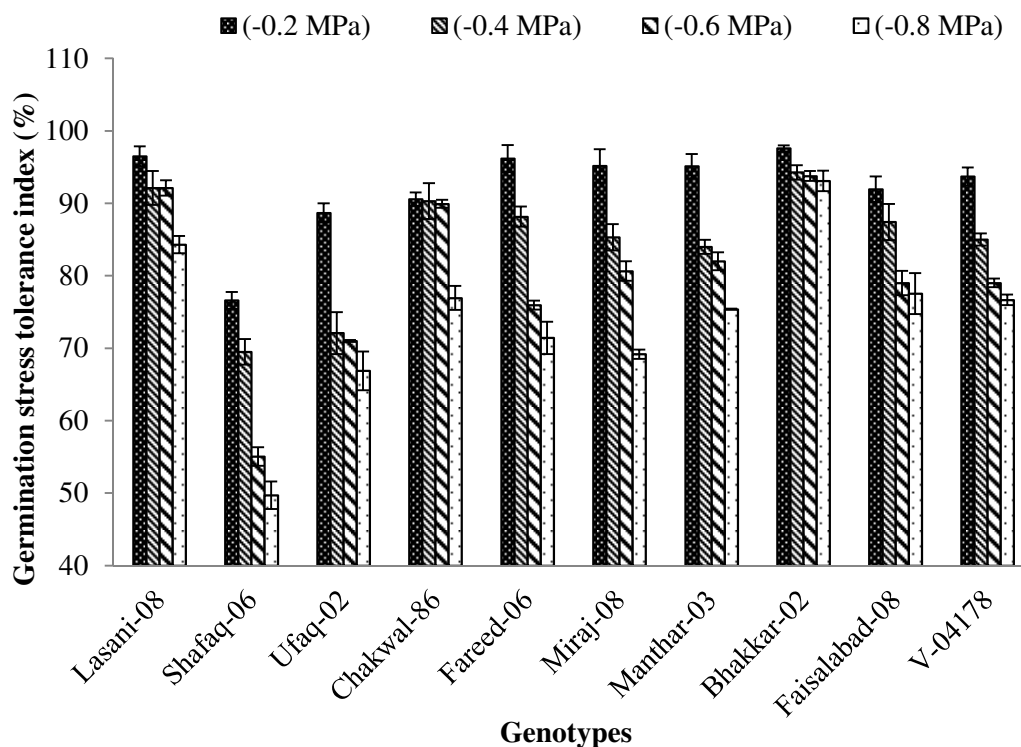


Fig. 4.4: Germination stress tolerance index of ten wheat (*Triticum aestivum* L.) genotypes under PEG (6000) induced water stress regimes (mean values $\pm$ S.E).

4.2. Laboratory Experiment-2

4.2.1. Seedling growth

Analysis of variance showed highly significant difference ($P \leq 0.001$) among genotypes and water stress treatments for RLSI, PHSI and DMSI (Figs. 4.5-4.7). In all tested genotypes maximum PHSI and RLSI was calculated in Bhakkar-02 (95.74%), (148.81%). Plant height stress tolerance index (PHSI) in Bhakkar-02 was statistically at par with Lasani-08 (93.48%) under water stress conditions. The maximum RLSI was recorded in Bhakkar-02 (146.05%) under water stress conditions. Minimum PHSI was calculated in Shafaq-06 (75.32%) and RLSI in Faisalabad (99.27%).

Maximum DMSI was recorded in Chakwal-86 (88.88%) which was statistically at par with Bhakkar-02 (88.0 %). Minimum DMSI was recorded in Shafaq-06 (69.64%) under water stress condition. Maximum relative root:shoot was calculated in Ufaq-02 (55.32%) under water stress conditions and minimum relative root:shoot was recorded in Lasani-08 (35.17%) in control treatment. Relative root:shoot of Ufaq-02 (55.32%) was statistically at par with Bhakkar-02 (55.22%) under water stress conditions (Fig. 4.8).

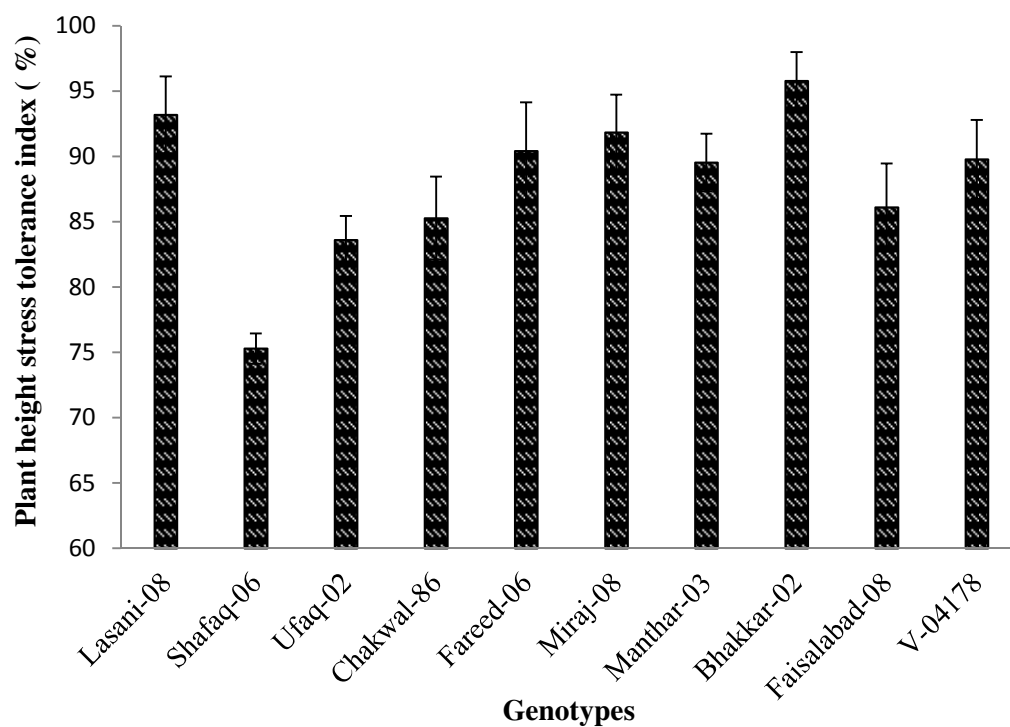


Fig. 4.5: Plant height stress tolerance index (PHSI) of ten wheat (*Triticum aestivum* L.) genotypes under water stress regimes (mean values $\pm$ S.E).

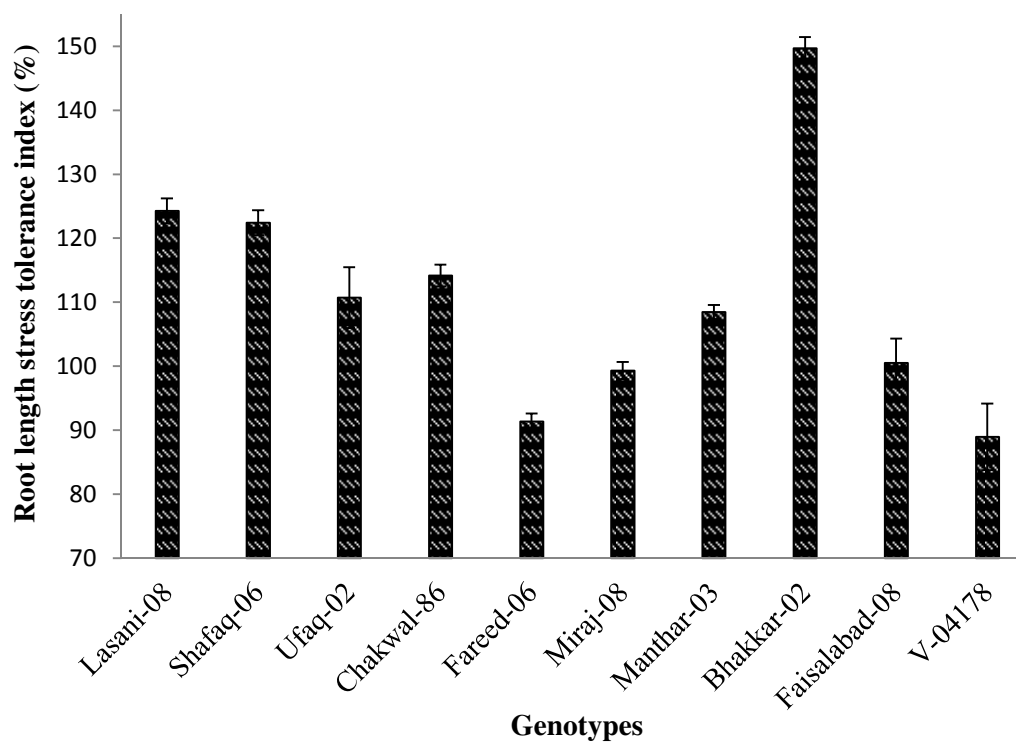


Fig. 4.6: Root length stress tolerance index (RLSI) of ten wheat (*Triticum aestivum* L.) genotypes under water stress regimes (mean values $\pm$ S.E).

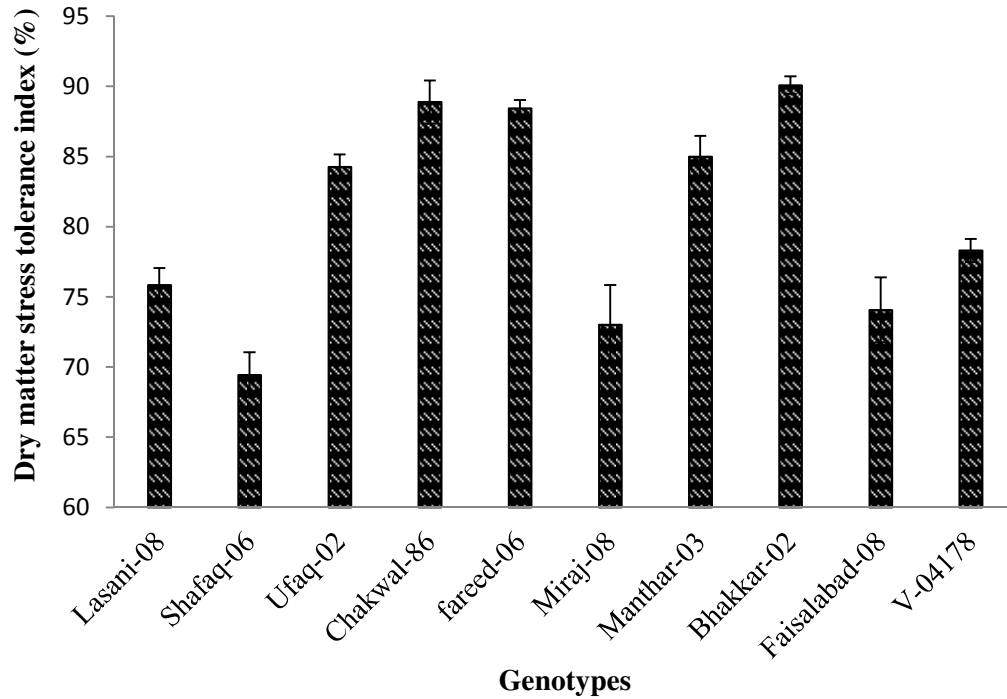


Fig. 4.7: Dry matter stress tolerance index (DMSI) of ten wheat (*Triticum aestivum* L.) genotypes under water stress regimes (mean values $\pm$ S.E.).

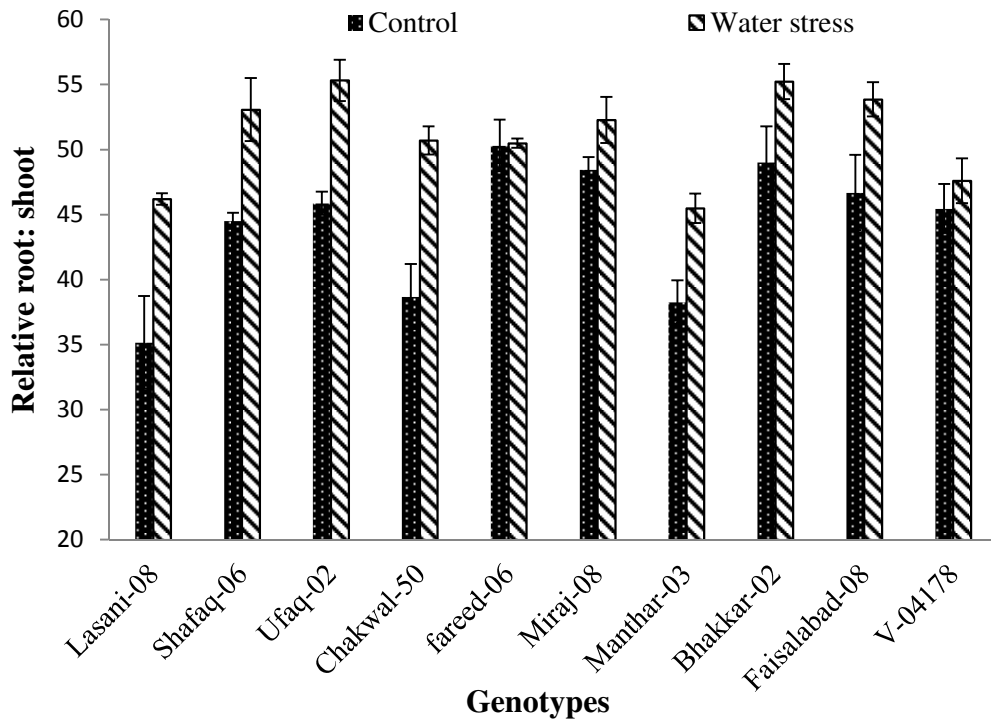


Fig. 4.8: Relative root: shoot of ten wheat (*Triticum aestivum* L.) genotypes under water stress regimes (mean values $\pm$ S.E.).

4.3. Glass House Experiment

Analysis of variance showed that values of genotypes and treatments and the interaction between G x F were different statistically ($P \leq 0.01$) for PHSI, and RLSI. Foliar application of nutrients (N, P, K alone or in different combinations) significantly increased the recorded parameters under water stress conditions.

4.3.1. Plant height stress tolerance index (PHSI)

Plant height stress tolerance index (PHSI) significantly affected under water stress conditions. Foliar application of N, P, and K alone or in different combinations was statistically highly significant for PHSI. Highest value for PHSI (93.28%), (87.97%) was recorded in both wheat cultivars with foliar application of NPK under water stress conditions, while minimum value (33.05%) of PHSI was recorded in Shafaq-06 at water spray treatment under water limited conditions (Fig 4.9).

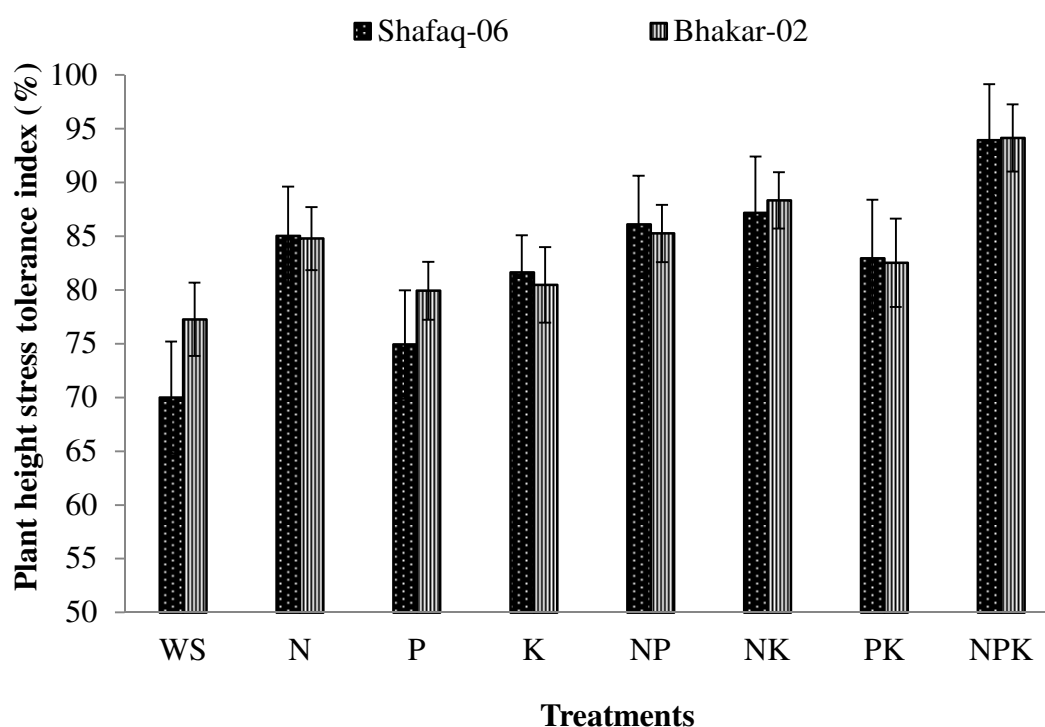


Fig. 4.9: Plant height stress tolerance index (PHSI) of wheat (*Triticum aestivum* L.) when plants were foliarly applied with various NPK levels under drought stress conditions(mean values $\pm$ S.E).

4.3.2. Root length stress tolerance index (RLSI)

Analysis of variance for the data for root length stress tolerance index revealed highly significant ($P \leq 0.001$) interaction between G x F. Both wheat Bhakkar-02 and Shafaq-06 maintained the highest values for RLSI (419.72%), (405.90%) with foliar applied NPK in combination under water stress conditions (Fig 4.10).

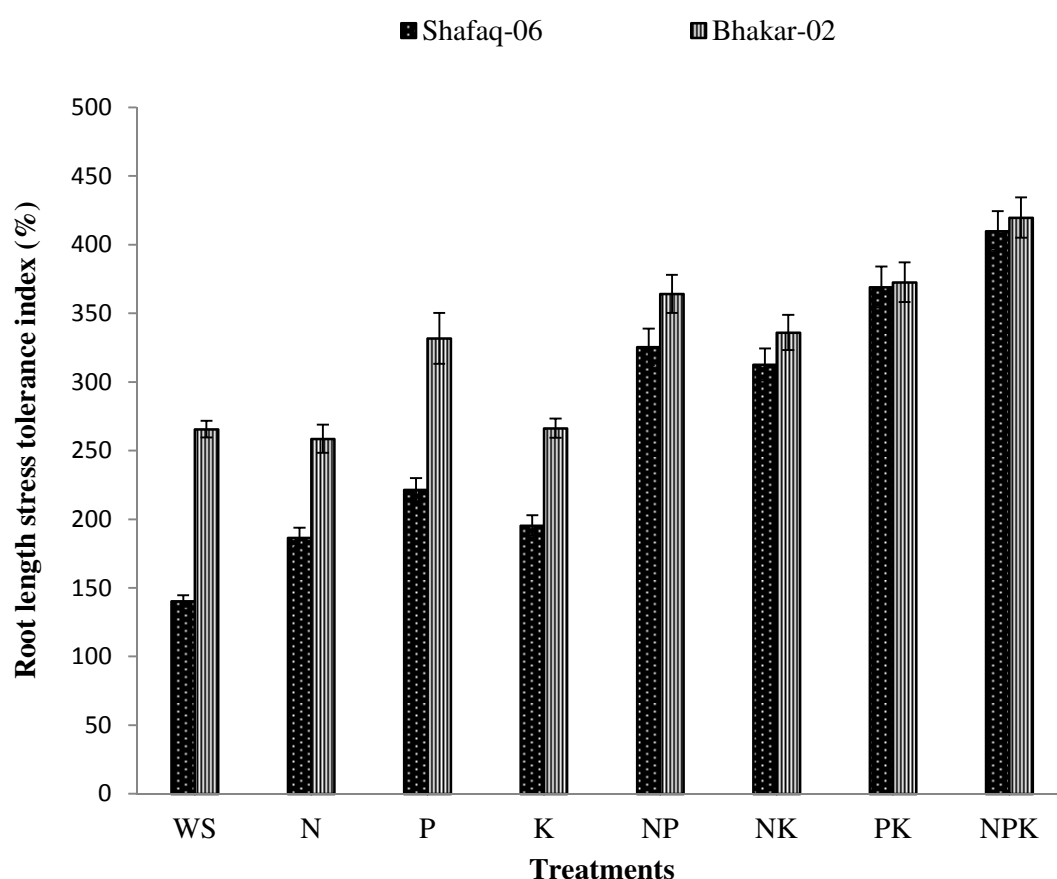


Fig. 4.10: Root length stress tolerance index (RLSI) of wheat (*Triticum aestivum* L.) when plants were foliarly applied with various NPK levels under drought stress conditions (mean values $\pm$ S.E).

4.3.3. Dry matter stress tolerance index (DMSI)

Dry matter stress tolerance index (DMSI) significantly decreased under water stress conditions. Application of NPK in combination had significant effect on the DMSI under water stress conditions (Figure 11). Maximum value (89.79%) for DMSI was recorded in NPK treatment while minimum value (37.24%) was obtained in water spray treatment (Fig 4.11).

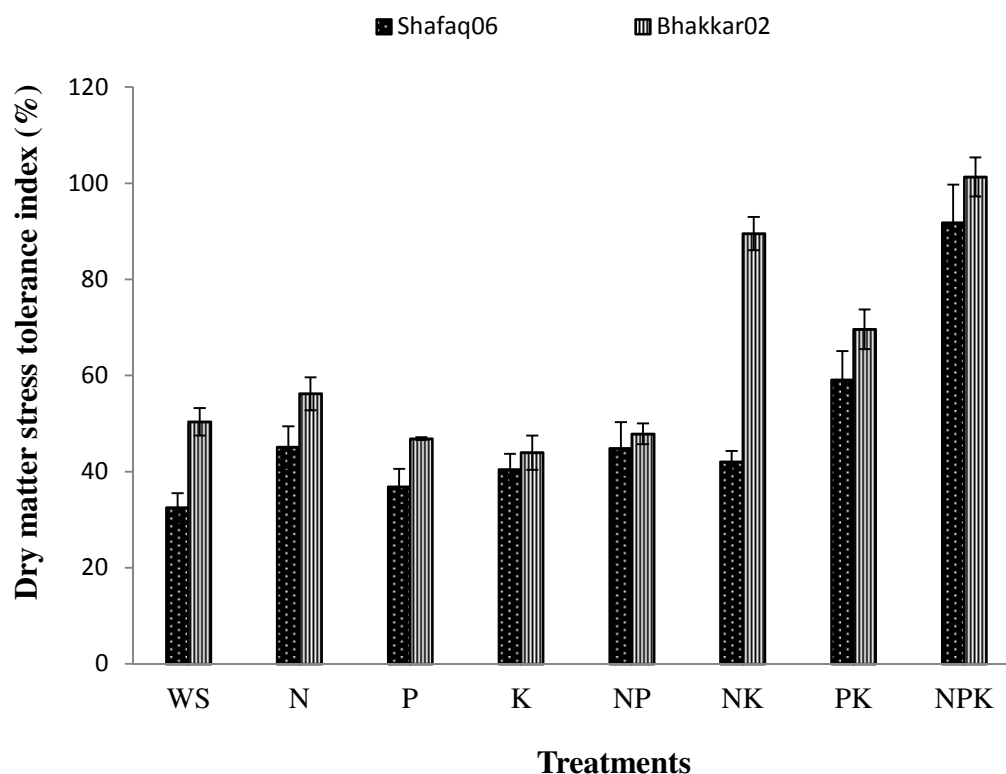


Fig. 4.11: Dry matter stress tolerance index (DMSI) of wheat (*Triticum aestivum* L.) when plants were foliarly applied with various NPK levels under drought stress conditions (mean values $\pm$ S.E).

4.3.4. Net CO₂ Assimilation Rate (*P_n*)

Wheat plants exhibited significantly lower ($P \leq 0.001$) *P_n* under water stress as compared to well-watered conditions (Table 4.1). The exposure to drought stress decreased *P_n* rate by 50% as compared to well-watered conditions. Analysis of variance for the data regarding *P_n* rate showed highly significant ($P \leq 0.001$) difference between genotypes. The plants of Bhakkar-02 maintained significantly higher *P_n* rate than Shafaq-06. The foliar spray of NPK significantly ($P \leq 0.001$) increased *P_n* rate in wheat plants and gave maximum value for this variable which is statistically at par with NK treatment whereas no NPK spray resulted in minimum *P_n* rate (Fig 4.12).

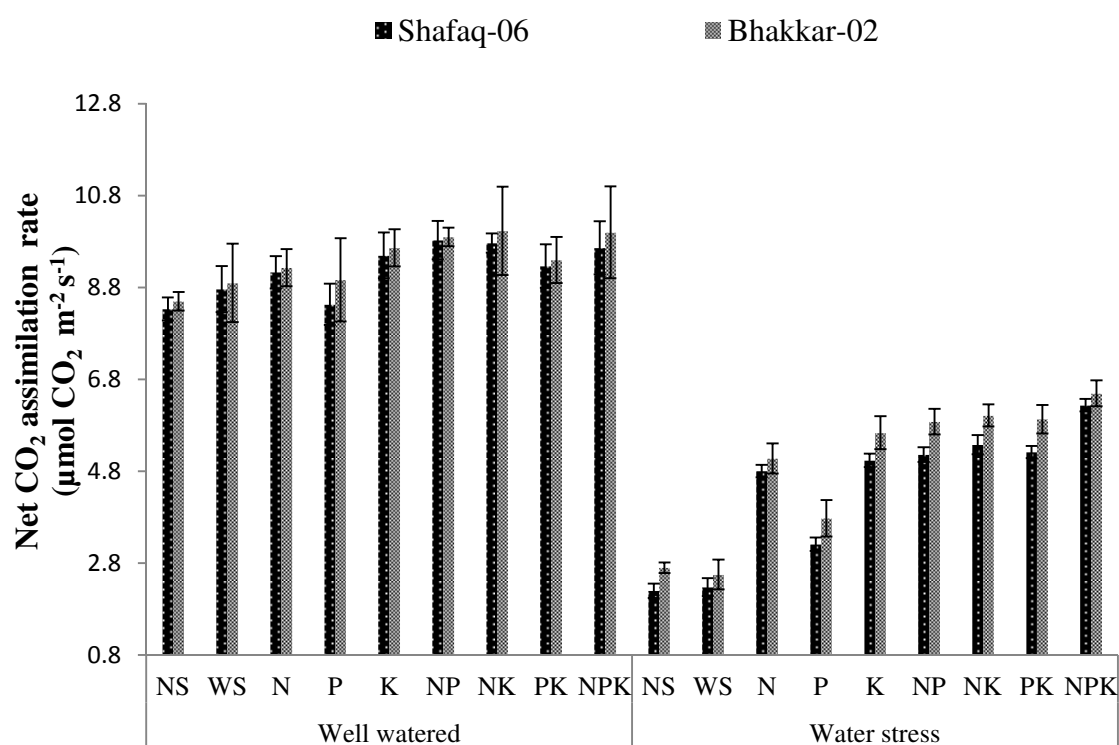


Fig. 4.12: Effect of supplemental foliar application of N, P, and K alone and in different combination on photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of two wheat genotypes in well-watered and water stress conditions (mean values $\pm$ S.E).

4.3.5. Stomatal conductance (g_s)

The data regarding g_s showed that water stress had highly significant ($P \leq 0.001$) on g_s . The exposure to drought stress decreased g_s (30%) as compare to normally grown wheat plants (Table 4.1). Analysis of variance for the data of g_s showed significant ($P \leq 0.05$) difference for genotypes (Table 4.1). Drought tolerant genotype (Bhakkar-02) gave higher value for g_s as compare to drought sensitive genotype (Shafaq-06). Foliar application of NPK significantly ($P \leq 0.001$) increased g_s in wheat plants which were statistically at par with NK, PK, K and N foliar sprays while minimum was recorded in no spray treatment (Fig 4.13). The interactions between WXF and GXW were significant. All other interactions were non-significant (Table 4.1).

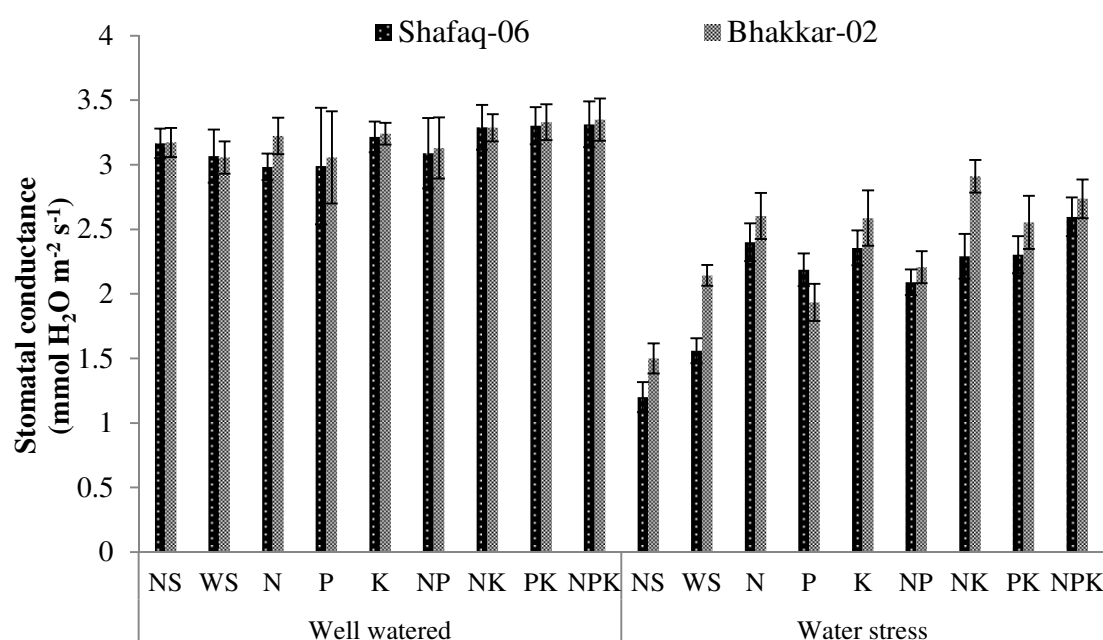


Fig. 4.13: Effect of supplemental foliar application of N, P, and K alone and in different combination on stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) of two wheat genotypes in well-watered and water stress conditions (mean values $\pm$ S.E).

4.3.6. Transpiration (*E*)

The data regarding *E* showed that water stress had highly significantly ($P<0.001$) effect on *E* (Table 4.1). A significant decrease of 44% was recorded in water stressed plants than well-watered ones. It was observed that Bhakkar-02 genotype maintained significantly higher (7%) *E* than, Shafaq-06 genotype (Table 4.1). Highly significant ($P<0.001$) effect of NPK application was observed on *E* of wheat plants. The plants foliarly sprayed with NPK gave maximum value than no sprayed plants (Fig 4.14). The interaction between WXF was significant. All other interactions were non-significant (Table 4.1).

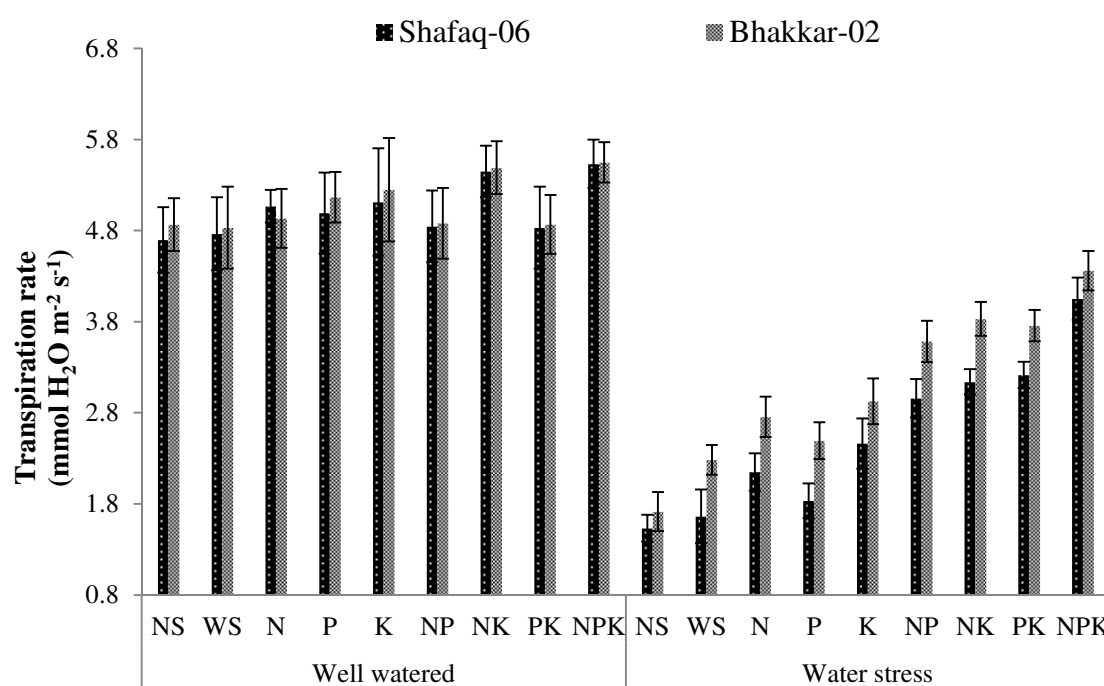


Fig. 4.14: Effect of supplemental foliar application of N, P, and K alone and in different combination on transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) of two wheat genotypes in well-watered and water stress conditions (mean values $\pm$ S.E).

4.3.7. Leaf water potential (Ψ_w)

The plants exposed to water stress showed a significant ($P<0.05$) decrease in Ψ_w (Table 4.1). Drought stress reduced Ψ_w by 39% with respect to well-watered conditions. The decrease in Ψ_w was more pronounced in Shafaq-06 than Bhakkar-02 genotype. A highly significant difference ($P<0.001$) was observed between foliar spray treatments. The application of NPK as foliar spray increased Ψ_w of plants and gave significantly higher as compared to water spray (Fig 4.15). The interaction between WXF was significant. All other interactions were non-significant (Table 4.1).

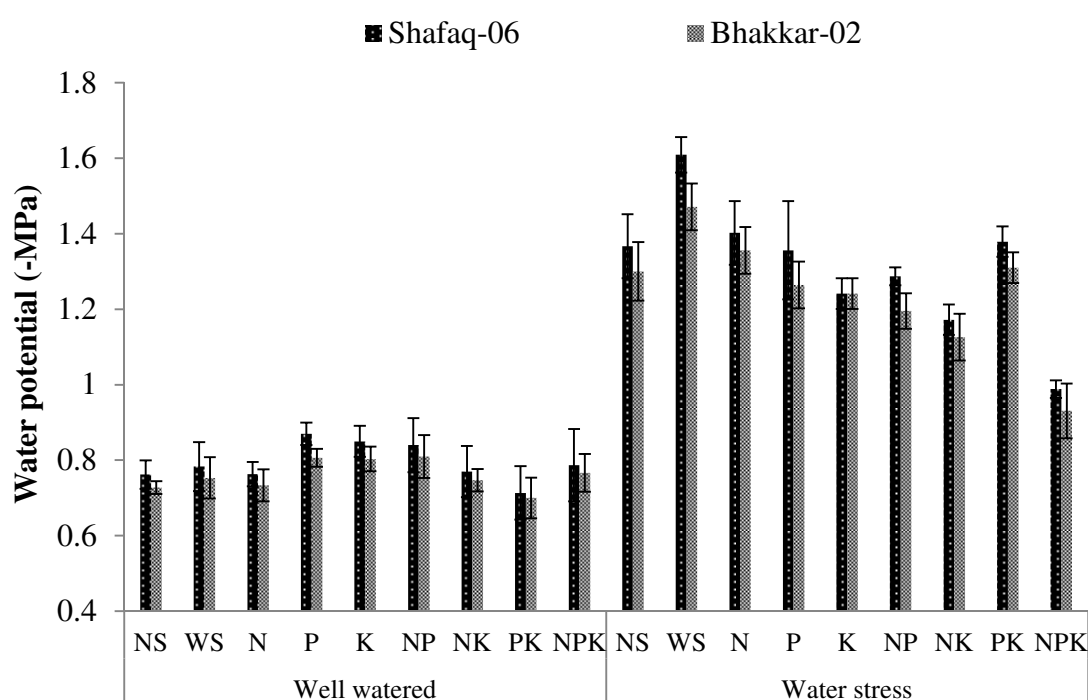


Fig. 4.15: Effect of supplemental foliar application of N, P, and K alone and in different combination on water potential (-MPa) of two wheat genotypes in well-watered and water stress conditions (mean values $\pm$ S.E).

4.3.8. Leaf osmotic potential (Ψ_s)

Analysis of variance for the data showed highly significant ($P<0.001$) reduction in Ψ_s of water stressed plants (Table 4.1). The water deficit conditions decreased Ψ_s by 8% and gave significantly lower for this variable than normal conditions (Fig 4.16). A much higher reduction (14%) was recorded with foliar N spray than no spray treatment (Table 4.1). The genotypes did not differ significantly for Ψ_s (Table 4.1). All interactions were non-significant.

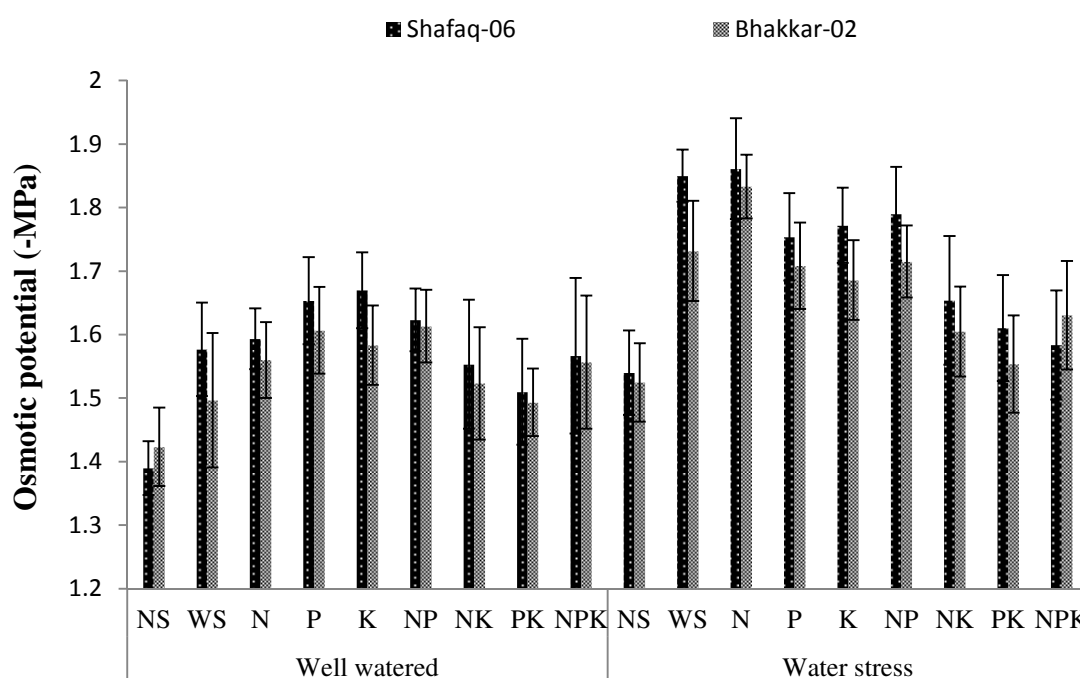


Fig. 4.16: Effect of supplemental foliar application of N, P, and K alone and in different combination on osmotic potential (-MPa) of two wheat genotypes in well-watered and water stress conditions (mean values $\pm$ S.E).

4.3.9. Leaf turgor potential (Ψ_p)

The data regarding Ψ_p revealed highly significant effect of drought stress on this variable. The limited water supply reduced Ψ_p by 47% as compared to well-watered conditions (Fig 4.17). The plants maintained significantly higher Ψ_p with foliar NPK spray, which was statistically at par with NP, N, K, and NK spray while lower Ψ_p was recorded in no spray treatment (Table 4.1). The genotypes did not differ significantly for Ψ_s . The interaction between WXF was significant. All other interactions were non-significant (Table 4.1).

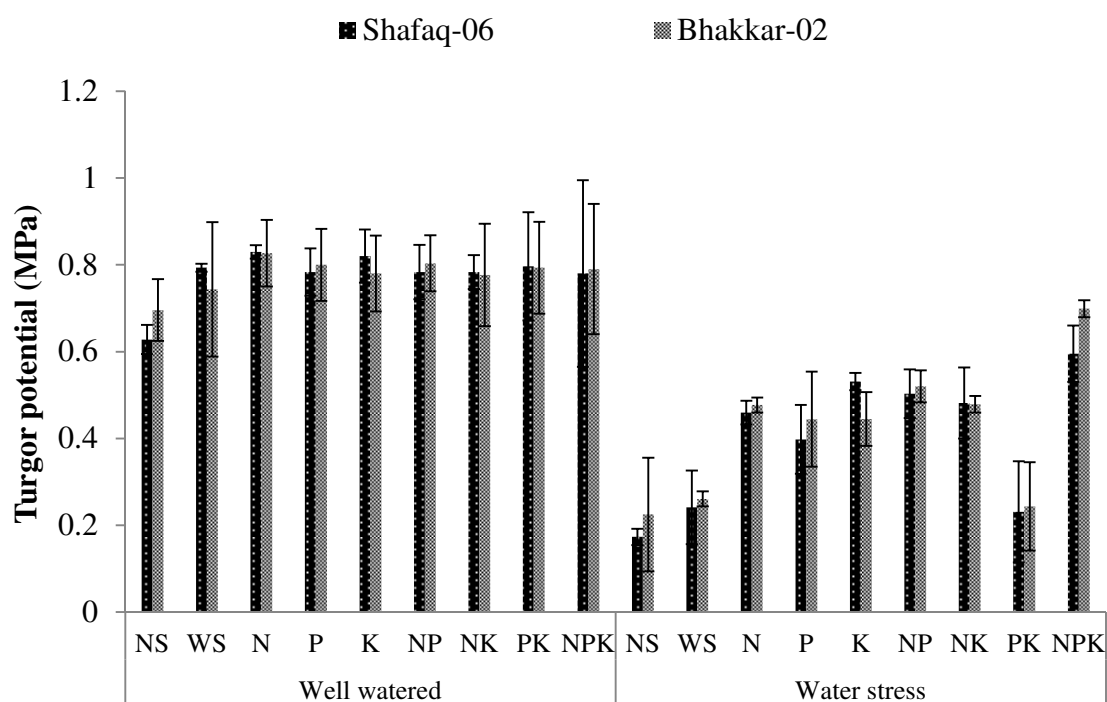


Fig. 4.17: Effect of supplemental foliar application of N, P, and K alone and in different combination on turgor potential (MPa) of two wheat genotypes in well-watered and water stress conditions (mean values $\pm$ S.E).

4.3.10. Leaf Nitrogen

Wheat plants exhibited significantly lower ($P \leq 0.001$) leaf N content under water stress as compare to well-watered conditions (Table 4.1). The exposure to drought stress decreased leaf nitrogen content by 11% as compared to normal supply of water. Analysis of variance for the data regarding leaf N content showed highly significant ($P \leq 0.001$) difference between genotypes. The plants of Bhakkar-02 maintained significantly higher leaf N content than Shafaq-06 (Table 4.1). The foliar spray of NPK significantly ($P \leq 0.001$) increased leaf N content in wheat plants and gave maximum value for this variable which was statistically at par with NK and NP spray whereas no NPK supply resulted in minimum leaf N content (Fig 4.18). The interactions between WXF and GXW were significant. All other interactions were non-significant (Table 4.1).

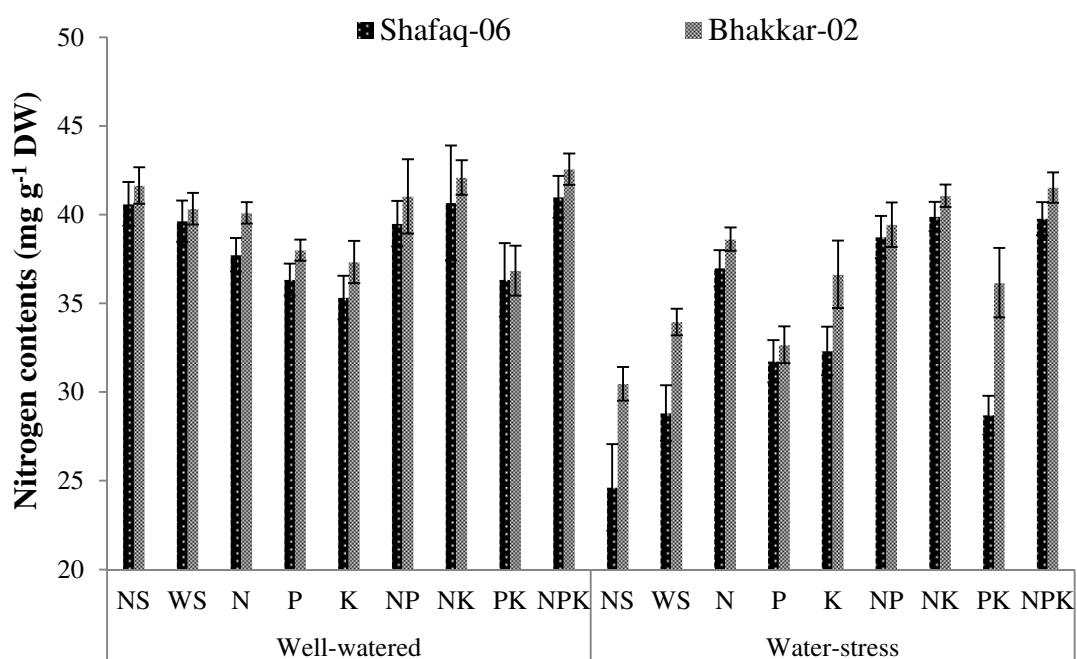


Fig. 4.18: Effect of supplemental foliar application of N, P, and K alone and in different combination on nitrogen contents (mg g⁻¹ DW) of two wheat genotypes in well-watered and water stress conditions (mean values $\pm$ S.E).

4.3.11. Leaf Phosphorus

The data regarding leaf P contents revealed highly significant ($P \leq 0.001$) differences of drought stress on this variable. The limited water supply reduced leaf P contents 17% as compared to well-watered conditions. The foliar spray of NPK was highly effective in the uptake of higher leaf P (Fig 4.19). The plants foliarly applied with NPK gave significantly higher value that was statistically at par with PK spray and minimum was recorded in no spray and K spray treatment (Table 4.1). The genotypes did not differ significantly for leaf P contents. The interaction between WXF was significant. All other interactions were non-significant (Table 4.1).

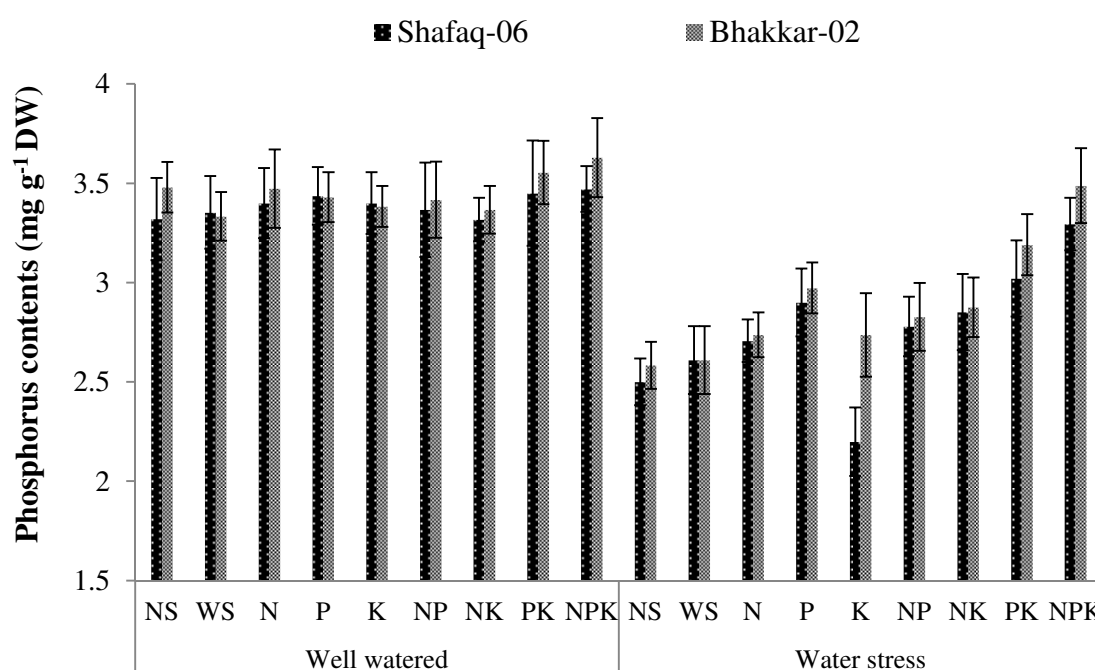


Fig. 4.19: Effect of supplemental foliar application of N, P, and K alone and in different combination on phosphorus contents ($\text{mg g}^{-1} \text{DW}$) of two wheat genotypes in well-watered and water stress conditions (mean values $\pm$ S.E).

4.3.12. Leaf Potassium

Wheat plants exhibited significantly higher ($P \leq 0.001$) leaf K content under water stress than normal conditions (Table 4.1). The exposure to drought stress increased leaf K content by 10% as compared to normal supply of water. The increased in leaf K content was more pronounced 9% in Bhakkar-02 than Shafaq-06 genotype (Fig 4.20). The plants maintained significantly higher (21%) leaf K content with foliar NPK spray as compare to no spray and P spray (Table 4.1). The interaction between GXW and GXF were significant. All other interactions were non-significant (Table 4.1).

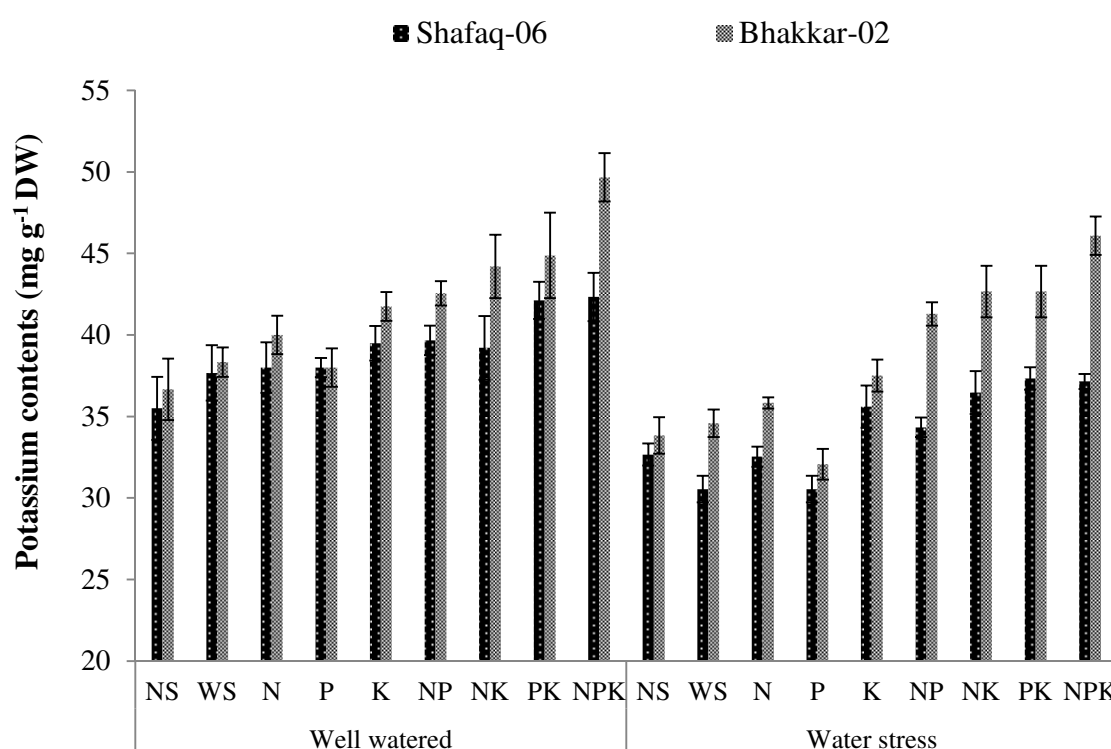


Fig. 4.19: Effect of supplemental foliar application of N, P, and K alone and in different combination on potassium contents ($\text{mg g}^{-1} \text{DW}$) of two wheat genotypes in well-watered and water stress conditions (mean values $\pm$ S.E).

Table 4.1: Mean parameter values of gas exchange, water relations, and nutrient content for main effects of genotype, foliar sprays, water levels and their interactions.

Parameter ^a	Genotype ^b		Foliar spray ^c									Water level ^d		Interactions				Cv ^e
	B	S	NS	WS	N	P	K	NP	NK	PK	NPK	WW	WS	GXW	GXF	WXF	GXWXF	
<i>Pn</i>	7.18a	6.79b	5.43 e	5.62 e	7.06 c	6.1 d	7.46 b	7.7 b	7.8 ab	7.46 b	8.1 a	9.29 a	4.65 b	NS	NS	***	NS	6.83
<i>E</i>	4.09a	3.8 b	3.2 g	3.39fg	3.73def	3.62 ef	3.94cde	4.07 cd	4.48 b	4.19 bc	4.88 a	5.06 a	2.82 b	**	NS	***	NS	11
<i>Gs</i>	2.78a	2.63b	2.26 d	2.46cd	2.8 ab	2.54 c	2.85 ab	2.63 bc	2.94 a	2.87 a	2.99 a	3.18 a	2.23 b	NS	NS	***	NS	9.62
Ψ_w	-1.0a	-1.1b	-1.04c	-1.15d	-1.06 c	-1.07cd	-1.03bc	-1.03bc	-0.95 b	-1.02bc	-0.87 a	-0.78a	-1.28b	NS	NS	***	NS	7.18
Ψ_s	-1.6a	-1.64a	-1.47 a	-1.7cd	-1.71 d	-1.68 d	-1.677cd	-1.69 d	-1.6bc	-1.5ab	-1.58bc	-1.55a	-1.69b	NS	NS	NS	NS	23.6
Ψ_p	0.6 a	0.59 a	0.43 d	0.51cd	0.65 a	0.61abc	0.64 a	0.65 a	0.63ab	0.52bcd	0.72 a	0.78 a	0.41 b	NS	NS	*	NS	10.8
N	38.36a	36.04b	34.34c	35.68c	38.36 b	34.68c	35.40 c	39.68ab	40.93a	34.51 c	41.22a	39.28a	35.11b	*	NS	***	NS	6.18
P	3.17 a	3.08 a	2.97cd	2.98cd	3.08bcd	3.18bc	2.93 d	3.09bcd	3.1bcd	3.3 ab	3.47 a	3.42 a	2.83 b	NS	NS	*	NS	8.96
K	40.14a	36.62b	34.66f	35.28ef	36.59 e	34.64 f	38.59 d	39.46cd	40.63bc	41.75 b	43.8 a	40.44a	36.31b	*	**	NS	NS	5.62

^a *Pn*=photosynthetic rate ($\mu\text{mol m}^{-2}\text{s}^{-1}$), *E*= transpiration ($\text{mmol m}^{-2}\text{s}^{-1}$), *gs*= stomatal conductance ($\text{mmol m}^{-2}\text{s}^{-1}$), Ψ_w = water potential (-MPa), Ψ_s = osmotic potential (-MPa), Ψ_p = turgor potential (MPa), ^b mean values across two genotypes and two water levels; B= Bhakkar-02, S= Shafaq-06; ^c mean values across nine foliar spray treatments; NS= No spray, WS= Water spray, N= 1.5% urea, P= 2% KH_2PO_4 , K= 3% K_2SO_4 , NP= 50% N 50% P, NK= 50% N 50% K, PK= 50% P 50% K, NPK= 33% N 33% P 33% K; ^d mean values across two water levels; WW= Well-watered, WS= Water stress; ^e coefficient of variation; Statistically significant (insignificant) differences for each main factor are indicated by different lower case letters; non-significant, *, **, *** significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively.

4.4. Wire House Experiment

4.4.1. Net CO₂ Assimilation Rate (*Pn*)

The data regarding *Pn* showed that water stress had highly significant ($P \leq 0.01$) on *Pn*. The exposure to drought stress (60% FC) decreased *Pn* (35%) as compare to normally grown wheat plants (100% FC). The maximum value ($10.47 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) for *Pn* was recorded in normally grown plants while minimum ($6.49 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was recorded in water stressed plants (Appendix 4.1).

Analysis of variance for the data of *Pn* showed highly significant ($P \leq 0.01$) difference for genotypes (Table.4.2). Drought tolerant genotype (Bhakkar-02) gave higher value ($9.19 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) for *Pn* as compare to drought sensitive ($8.07 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) genotype (Shafaq-06) (Appendix 4.2). Foliar application of NPK significantly ($P \leq 0.05$) increased *Pn* ($9.07 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in wheat plants as compared to no spray ($8.19 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) treatment (Appendix 4.1). It was observed that plants maintained significantly higher (24%) *Pn* at tillering ($9.83 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) than, anthesis ($7.43 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) stage (Appendix 4.2).

The highly significant ($P \leq 0.001$) interaction (SXW) revealed that plants maintained higher *Pn* at tillering stage under normal condition whereas, exposure to drought stress at anthesis stage resulted in lowest for this variable (Fig.4.21). All other interactions were statistically non-significant.

4.4.2. Stomatal Conductance (*gs*)

Wheat plants exhibited significantly lower ($P \leq 0.001$) *gs* under water stress (60% FC) than normal conditions (100% FC) (Table 4.2). The exposure to drought stress reduced *gs* by 50% as compared to normal supply of water. The maximum value ($5.10 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) for *gs* was recorded in normally grown plants while minimum ($2.54 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) was recorded in water stressed plants (Appendix 4.3).

Analysis of variance for the data regarding *gs* showed highly significant ($P \leq 0.001$) difference between genotypes (Table.4.9). The plants of Bhakkar-02 maintained significantly higher *gs* ($4.05 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) than Shafaq-06 ($3.58 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) (Appendix 4.4). The foliar spray of NPK significantly ($P \leq 0.001$) increased *gs* in wheat

plants and gave maximum value ($4.13 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) for this variable whereas, no NPK application resulted in minimum g_s ($3.5 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) (Appendix 4.3). A highly significant difference was also observed between growth stages for this variable. The plants exhibited higher g_s at tillering ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) than, anthesis ($3.68 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) stage (Appendix 4.4).

The significant interaction between genotypes (G) and treatments (T) revealed that drought tolerant genotype (Bhakkar-02) exhibited higher g_s by foliar NPK spray, while no NPK application gave lowest for this variable in sensitive genotype (Shafaq-06) (Fig. 4.22). All other interactions were statistically non-significant (Table.4.9).

4.4.3. Transpiration Rate (E)

The data regarding E showed that water stress had highly significantly ($P \leq 0.01$) effect on E (Table.4.9). A significant decrease of transpiration rate (48%) was recorded in water stressed (60% FC) plants than normal ones (100% FC). It was observed that plants maintained significantly higher (18%) E at tillering ($3.62 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) than, anthesis ($3.08 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) stage (Appendix 4.6).

Highly significant ($P \leq 0.01$) effect of NPK application was observed on E of wheat plants. The plants foliarly sprayed with NPK gave maximum value ($3.91 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) than control (no NPK application) plants ($2.78 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) (Appendix 4.5). Wheat genotype Bhakkar-02 exhibited higher (11%) E than Shafaq-06.

The interaction GXT also showed highly significant ($P \leq 0.001$) differences for this variable. Drought tolerant genotype (Bhakkar-02) showed higher E by foliar spray of NPK than control plants of sensitive genotype (Shafaq-06) (Fig. 4.23).

The interaction TXW was also significant for E . The foliar spray of NPK resulted in maximum E under both normal and water stress conditions, whereas, minimum value was recorded in control plants (no spray) under drought stress (Fig. 4.23).

The highly significant ($P \leq 0.001$) interaction (SXW) revealed that plants maintained higher E at tillering stage under normal condition whereas, exposure to drought stress at tillering stage resulted in lowest for this variable (Fig. 4.23). All other interactions were non-significant (Table.4.9).

Table 4.2: Analysis of variance table for Net CO₂ assimilation rate (*Pn*), stomatal conductance (*gs*) and transpiration rate (*E*) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions with foliar applied nutrient NPK.

SOV	Net CO₂ assimilation rate (<i>Pn</i>)	Stomatal conductance (<i>gs</i>)	Transpiration rate (<i>E</i>)
Genotypes (G)	**	***	***
Treatment (T)	*	***	***
Stage (S)	***	*	***
Water levels (W)	***	***	***
GXT	NS	*	***
GXS	NS	NS	NS
GXW	NS	NS	NS
TXS	NS	NS	NS
TXW	NS	NS	***
SXW	***	NS	***
GXTXS	NS	NS	NS
GXTXW	NS	NS	NS
GXSXW	NS	NS	NS
TXSXW	NS	NS	NS
GXTXSXW	NS	NS	NS
CV ^a	13.17	9.81	5.59

*, **, *** = Significant at 0.05, 0.01 and 0.001 level

NS = Non significant respectively, ^a coefficient of variation.

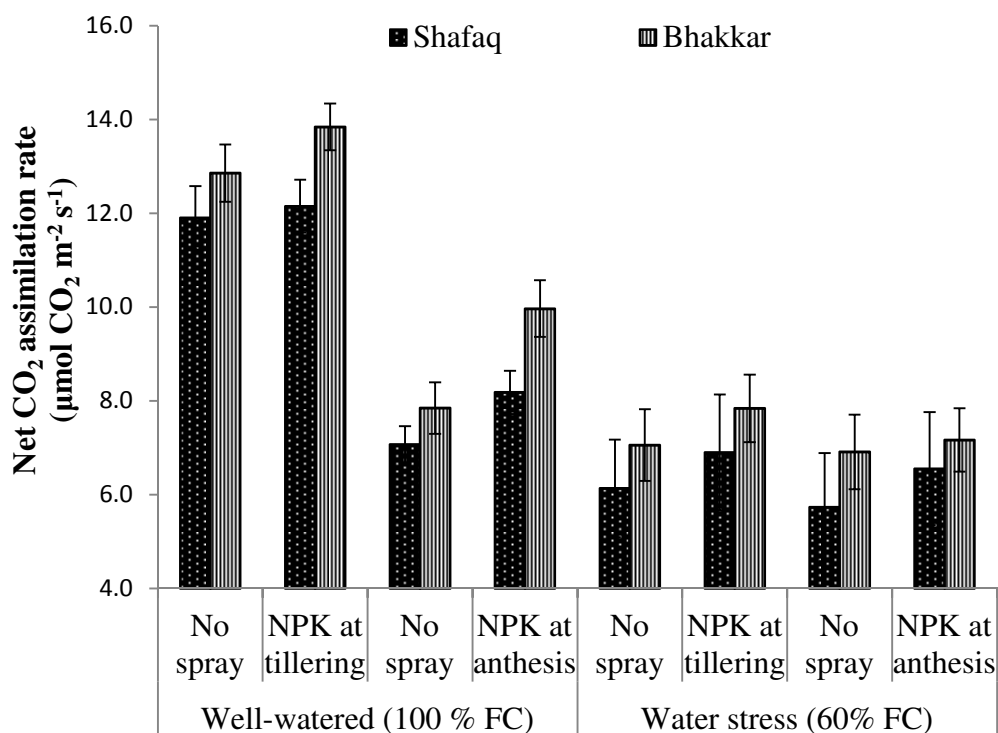


Fig. 4.21: Effect of supplemental foliar NPK application on Net CO₂ assimilation rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

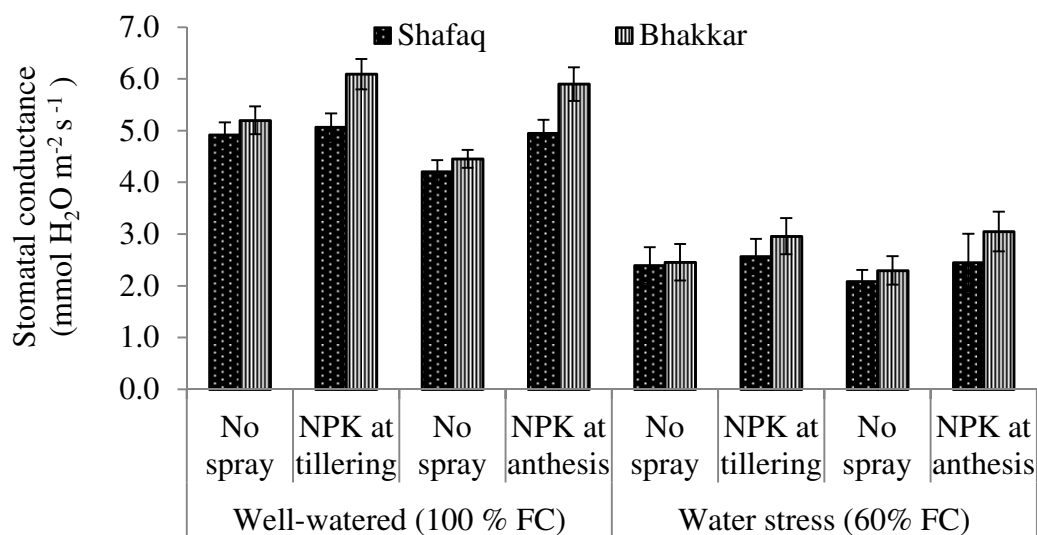


Fig. 4.22: Effect of supplemental foliar NPK application on stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

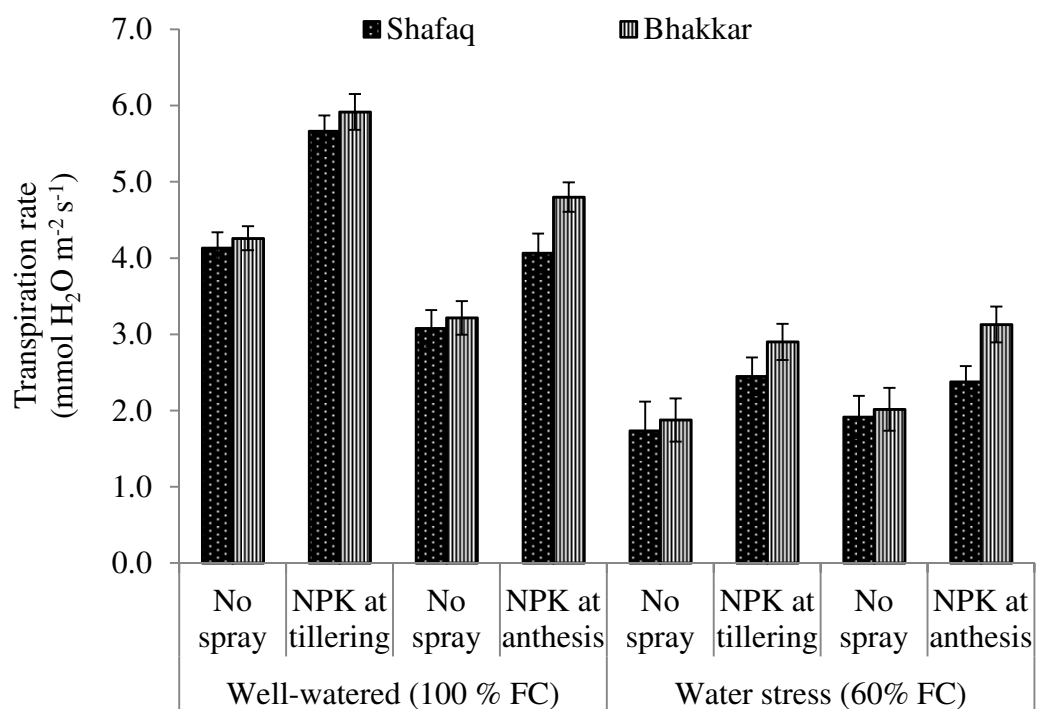


Fig. 4.23: Effect of supplemental foliar NPK application on transpiration rate ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

4.4.4. Leaf Water Potential (Ψ_w)

The plants exposed to water stress showed a significant ($P<0.001$) decrease in Ψ_w (Table 4.3). Drought stress reduced Ψ_w by 41% with respect to normal conditions. The maximum value (-0.84 MPa) was recorded in well-watered (100% FC) plant and minimum (-1.19 MPa) was recorded in water stressed (60% FC) wheat plants (Fig. 4.24). The decrease in Ψ_w was more pronounced at anthesis (-1.11 MPa) than tillering (-0.92 MPa) stage (Appendix 4.8).

Wheat genotypes (Bhakkar-02 and Shafaq-06) exhibited non-significant difference for Ψ_w . However, a significant difference ($P<0.05$) was observed between treatments. The application of NPK as foliar spray increased Ψ_w (10%) of plants and gave significantly higher value (-0.96 MPa) as compared to no spray (-1.07 MPa) (Appendix 4.7).

All the interactions among different factors were non-significant (Table 4.3).

4.4.5. Leaf Osmotic Potential (Ψ_s)

Analysis of variance for the data showed highly significant ($P<0.001$) reduction in Ψ_s of water stressed plants (Table 4.3). The water deficit conditions decreased Ψ_s by 7% and gave significantly lower value (-1.49 MPa) for this variable than normal (-1.38 MPa) conditions (Appendix 4.9).

A much higher reduction (27%) was recorded at anthesis (-1.60 MPa) than tillering (-1.27 MPa) stage (Appendix 4.10), (Fig. 4.25). The genotypes, treatments and all interactions did not differ significantly for Ψ_s (Table 4.3).

4.4.6. Leaf Turgor Potential (Ψ_p)

The data regarding Ψ_p revealed highly significant effect of drought stress on this variable. The limited water supply reduced Ψ_p by 44% as compared to normal conditions. The maximum value (0.54 MPa) was recorded in well-watered (100% FC) plant and minimum (0.31 MPa) was recorded in water stressed (60% FC) wheat plants. The plants maintained significantly higher (43%) Ψ_p at anthesis (0.50 MPa) than tillering (0.35 MPa) stage (Appendix 4.12).

The genotypes also varied significantly for this variable. Wheat genotype Bhakkar-02 (0.45 MPa) exhibited higher Ψ_p than Shafaq-06 (0.39 MPa). The foliar spray

of NPK was highly effective in the maintenance of Ψ_p . The plants foliarly applied with NPK gave significantly higher value (0.47 MPa) than those with no spray (0.37 MPa) (Appendix 4.11).

The significant interaction TXS revealed that foliar spray of NPK at anthesis stage resulted in maximum Ψ_p in plants whereas, no spray at tillering stage gave minimum value for this variable (Fig. 4.26).

The interaction between growth stages and water stress levels (SXW) was also significant ($P \leq 0.05$) for Ψ_p . The highest Ψ_p was recorded at anthesis stage in normal plants whereas, water stressed plants gave the lowest value for this variable at tillering stage (Fig. 4.26). All other interactions were non-significant for Ψ_p (Table 4.3).

4.4.7. Leaf Relative Water Contents (RWC)

Analysis of variance regarding RWC revealed that it was significantly reduced ($P < 0.01$) in water stressed wheat plants as compared to non-stressed plants (Table 4.3). Drought stress caused a (15%) reduction in RWC as compared to normal plants of both wheat genotypes i.e. Bhakkar-02 and Shafaq-06. The maximum value (88.48 %) was recorded in well-watered (100% FC) plant and minimum (75.18 %) was recorded in water stressed (60% FC) wheat plants (Fig. 4.27). A more prominent decrease was recorded at anthesis (78.47%) as compared to tillering stage (85.19%), (Appendix 4.14).

Analysis of variance for the data regarding RWC showed highly significant ($P \leq 0.01$) difference between genotypes. The plants of Bhakkar-02 maintained significantly higher RWC (85.41 %) than Shafaq-06 (78.24 %), (Appendix 4.14). The foliar spray of NPK significantly ($P \leq 0.01$) increased RWC in wheat plants and gave maximum value (85.04 %) for this variable whereas, no NPK spray (78.62 %) resulted in minimum RWC (Appendix 4.13).

All interactions were non-significant for RWC (Table 4.3).

Table 4.4: Analysis of variance tables for water potential, osmotic potential (-MPa), turgor potential (MPa) and relative water contents (%) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions with foliar applied nutrient NPK.

SOV	Water potential (-Mpa)	Osmotic potential (-Mpa)	Turgor potential (Mpa)	Relative water contents (%)
Genotypes (G)	NS	NS	**	**
Treatment (T)	*	NS	***	**
Stage (S)	***	***	***	**
Water levels (W)	***	*	***	***
GXT	NS	NS	NS	NS
GXS	NS	NS	NS	NS
GXW	NS	NS	NS	NS
TXS	NS	NS	**	NS
TXW	NS	NS	NS	NS
SXW	NS	NS	*	NS
GXTXS	NS	NS	NS	NS
GXTXW	NS	NS	NS	NS
GXSXW	NS	NS	NS	NS
TXSXW	NS	NS	NS	NS
GXTXSXW	NS	NS	NS	NS
CV ^a	16.10	12.17	14.29	9.62

*, **, *** = Significant at 0.05, 0.01 and 0.001 level

NS = Non significant respectively, ^a coefficient of variation.

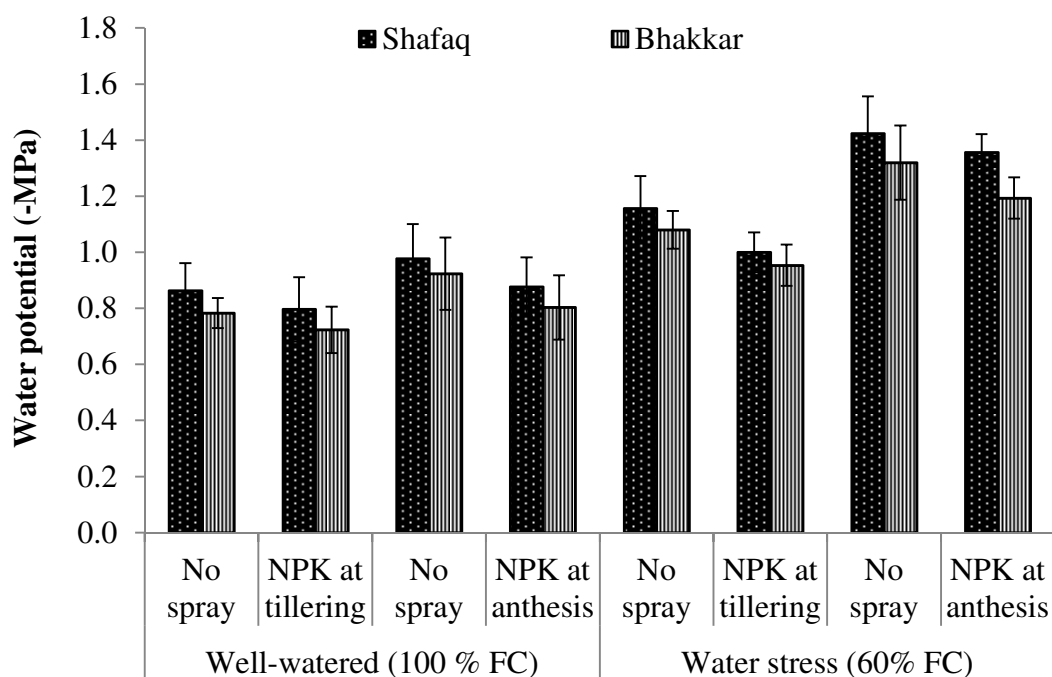


Fig. 4.24: Effect of supplemental foliar NPK application on water potential (-MPa) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

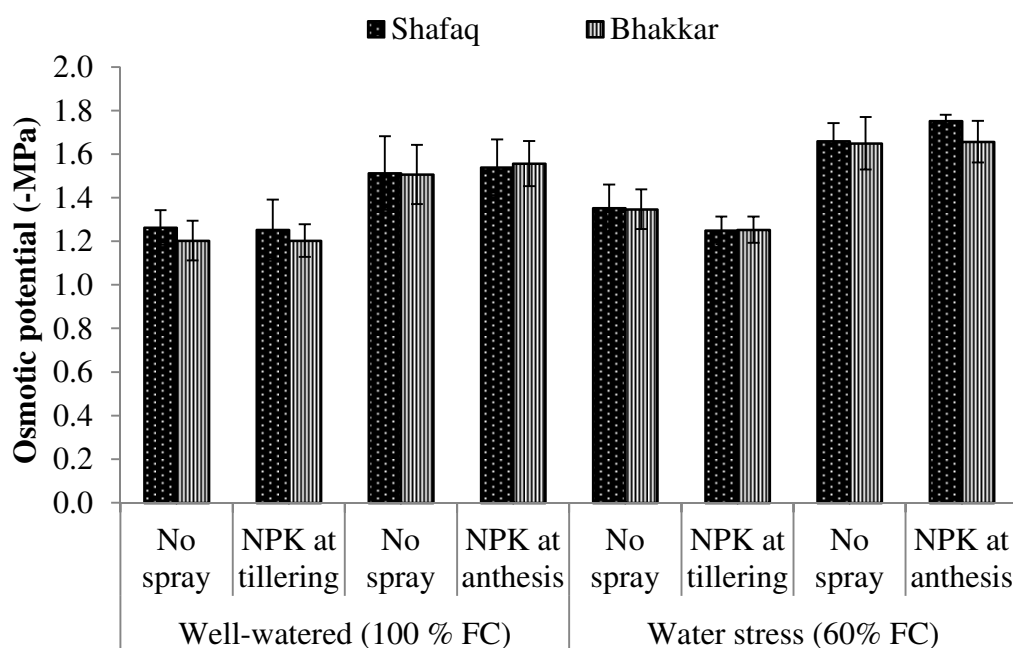


Fig. 4.25: Effect of supplemental foliar NPK application on osmotic potential (-MPa) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

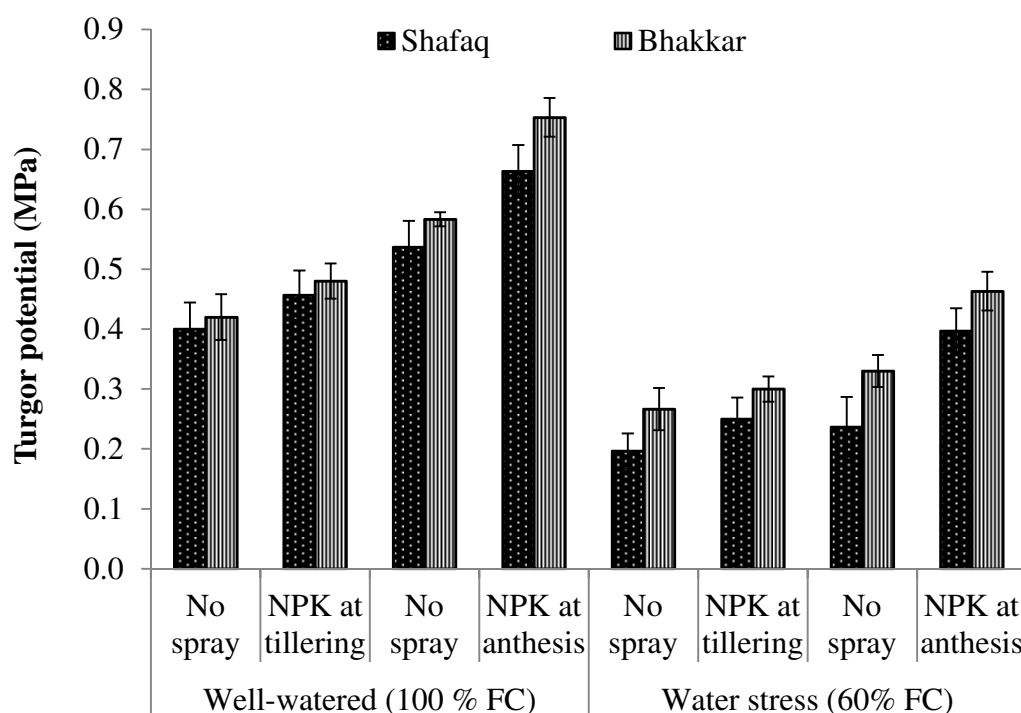


Fig. 4.26: Effect of supplemental foliar NPK application on turgor potential (MPa) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

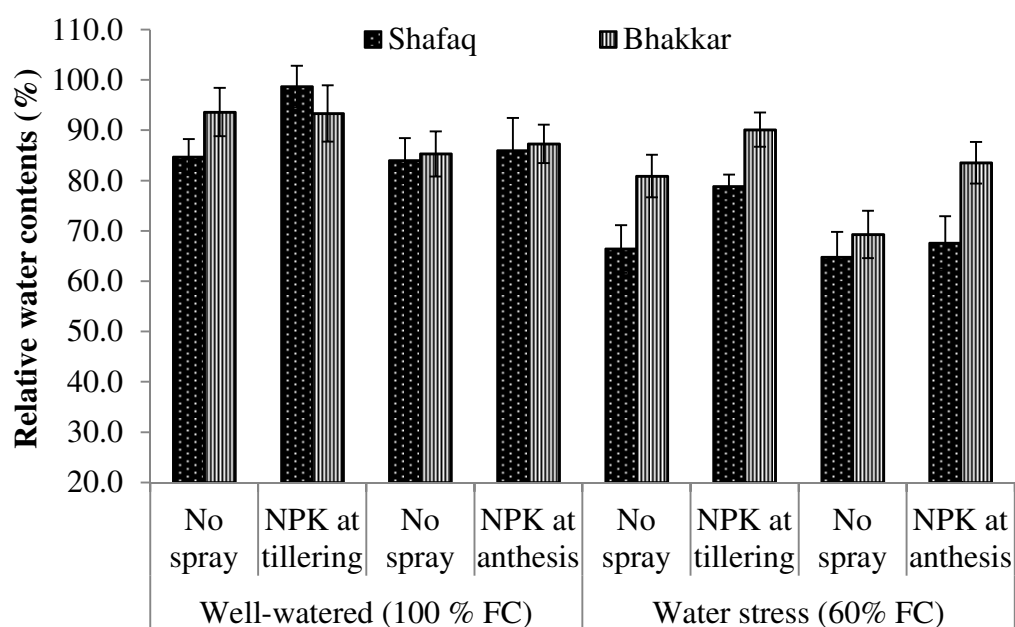


Fig. 4.27: Effect of supplemental foliar NPK application on relative water contents (%) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

4.4.8. Leaf Chlorophyll (a) Contents

Wheat genotypes (Bhakkar-02 and Shafaq-06) exhibited non-significant difference for chlorophyll (a) contents. However, highly significant difference ($P \leq 0.001$) was observed between treatments (Table 4.4). The application of NPK as foliar spray increased chlorophyll (a) contents (13%) of plants and gave significantly higher value ($1.33 \text{ mg g}^{-1} \text{ FW}$) as compared to no spray ($1.15 \text{ mg g}^{-1} \text{ FW}$) treatment (Appendix 4.15), (Fig. 4.28). The water levels also showed non-significant difference for this variable (Table 4.4).

A much higher reduction (31%) was recorded at anthesis ($1.01 \text{ mg g}^{-1} \text{ FW}$) than tillering ($1.46 \text{ mg g}^{-1} \text{ FW}$) stage (Appendix 4.16). All interactions were non-significant for chlorophyll (a) contents (Table 4.4).

4.4.9. Leaf Chlorophyll (b) Contents

The data regarding chlorophyll (b) contents revealed highly significant effect of drought stress on this variable (Table 4.4). The limited water supply reduced chlorophyll (b) contents by 15% as compared to normal conditions. The maximum value ($0.67 \text{ mg g}^{-1} \text{ FW}$) for chlorophyll b contents was recorded in normally grown plants while minimum ($0.57 \text{ mg g}^{-1} \text{ FW}$) was recorded in water stressed plants (Appendix 4.17).

The genotypes also varied significantly for this variable (Table 4.4). Wheat genotype Bhakkar-02 exhibited higher chlorophyll (b) contents ($0.65 \text{ mg g}^{-1} \text{ FW}$) than Shafaq-06 ($0.59 \text{ mg g}^{-1} \text{ FW}$). The foliar spray of NPK was highly effective in the maintenance of chlorophyll (b) contents. The plants foliarly applied with NPK gave significantly higher value ($0.64 \text{ mg g}^{-1} \text{ FW}$) than those with no spray ($0.59 \text{ mg g}^{-1} \text{ FW}$). A much higher reduction was recorded at anthesis ($0.60 \text{ mg g}^{-1} \text{ FW}$) than tillering ($0.64 \text{ mg g}^{-1} \text{ FW}$) stage (Appendix 4.18).

The interaction between growth stages and water stress levels (SXW) was also significant ($P \leq 0.05$) for chlorophyll (b) contents. The highest chlorophyll (b) contents were recorded at tillering stage in normal plants whereas, water stressed plants gave the lowest value at anthesis stage for this variable (Fig. 4.29).

All other interactions were non-significant for chlorophyll (b) contents (Table 4.4).

4.4.10. Leaf Total Chlorophyll Contents

Wheat plants exhibited significantly lower ($P \leq 0.001$) leaf total chlorophyll contents under water stress (60% FC) than normal conditions (100% FC) (Table 4.4). The exposure to drought stress reduced the total chlorophyll contents (8%) as compared to normal supply of water. The maximum value ($1.94 \text{ mg g}^{-1} \text{ FW}$) for total chlorophyll contents were recorded in normally grown plants while minimum ($1.79 \text{ mg g}^{-1} \text{ FW}$) was recorded in water stressed plants (Appendix 4.19).

Analysis of variance for the data regarding total chlorophyll contents showed highly significant ($P \leq 0.001$) difference between genotypes. The plants of Bhakkar-02 maintained significantly higher total chlorophyll contents ($1.94 \text{ mg g}^{-1} \text{ FW}$) than Shafaq-06 ($1.79 \text{ mg g}^{-1} \text{ FW}$). The foliar spray of NPK significantly ($P \leq 0.001$) increased total chlorophyll contents in wheat plants and gave maximum value ($1.98 \text{ mg g}^{-1} \text{ FW}$) for this variable whereas, no NPK application resulted in minimum total chlorophyll contents ($1.75 \text{ mg g}^{-1} \text{ FW}$).

A highly significant difference was also observed between growth stages for this variable. The plants exhibited higher total chlorophyll contents at tillering ($2.10 \text{ mg g}^{-1} \text{ FW}$) than anthesis ($1.63 \text{ mg g}^{-1} \text{ FW}$) stage (Appendix 4.20).

The significant interaction between genotypes (G) and growth stages (S) and water levels (W) revealed that drought tolerant genotype (Bhakkar-02) exhibited higher total chlorophyll contents at tillering in well-watered (100% FC) conditions while drought sensitive genotype (Shafaq-06) gave lowest value in water stress (60% FC) conditions for this variable (Fig. 4.30).

All other interactions were non-significant (Table 4.4).

4.4.11. Leaf Total Carotenoid Contents

Analysis of variance regarding leaf total carotenoid contents revealed that it was significantly reduced ($P<0.01$) 7% at anthesis stage than of tillering stage (Appendix 4.22).

Water levels (100% FC and 60% FC) exhibited non-significant difference for leaf total carotenoid contents. However, highly significant difference ($P\leq 0.001$) was observed between treatments. The foliar spray of NPK significantly ($P\leq 0.001$) increased leaf total carotenoid contents in wheat plants and gave maximum value ($0.45 \text{ mg g}^{-1} \text{ FW}$) for this variable whereas, no NPK spray ($0.40 \text{ mg g}^{-1} \text{ FW}$) resulted in minimum leaf total carotenoid contents (Appendix 4.21).

Analysis of variance for the data regarding leaf total carotenoid contents showed highly significant ($P\leq 0.01$) difference between genotypes. The plants of Bhakkar-02 maintained significantly higher ($0.44 \text{ mg g}^{-1} \text{ FW}$) leaf total carotenoid contents than Shafaq-06 ($0.41 \text{ mg g}^{-1} \text{ FW}$).

Interaction between GXW leaf total carotenoid contents revealed significant ($P\leq 0.05$) difference. The highest value ($0.45 \text{ mg g}^{-1} \text{ FW}$) was recorded in drought tolerant Bhakkar-02 in normal (100% FC) plants whereas, water stressed (60% FC) plants gave the minimum value ($0.39 \text{ mg g}^{-1} \text{ FW}$) for this variable (Fig. 4.31).

All other interactions were non-significant for leaf total carotenoid contents (Table 4.4).

Table 4.4: Analysis of variance tables for chlorophyll a, chlorophyll b, total chlorophyll contents and total carotenoid contents (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions with foliar applied nutrient NPK.

SOV	Chlorophyll a (mg g⁻¹ FW)	Chlorophyll b (mg g⁻¹ FW)	Total chlorophyll (mg g⁻¹ FW)	Total carotenoids (mg g⁻¹ FW)
Genotypes (G)	NS	***	***	**
Treatment (T)	***	***	***	***
Stage (S)	***	**	***	**
Water levels (W)	NS	***	***	NS
GXT	NS	NS	NS	NS
GXS	NS	NS	NS	NS
GXW	NS	NS	NS	*
TXS	NS	NS	NS	NS
TXW	NS	NS	NS	NS
SXW	NS	*	NS	NS
GXTXS	NS	NS	NS	NS
GXTXW	NS	NS	NS	NS
GXSXW	NS	NS	*	NS
TXSXW	NS	NS	NS	NS
GXTXSXW	NS	NS	NS	NS
CV ^a	12.96	7.20	5.02	8.85

*, **, *** = Significant at 0.05, 0.01 and 0.001 level

NS = Non significant respectively, ^a coefficient of variation.

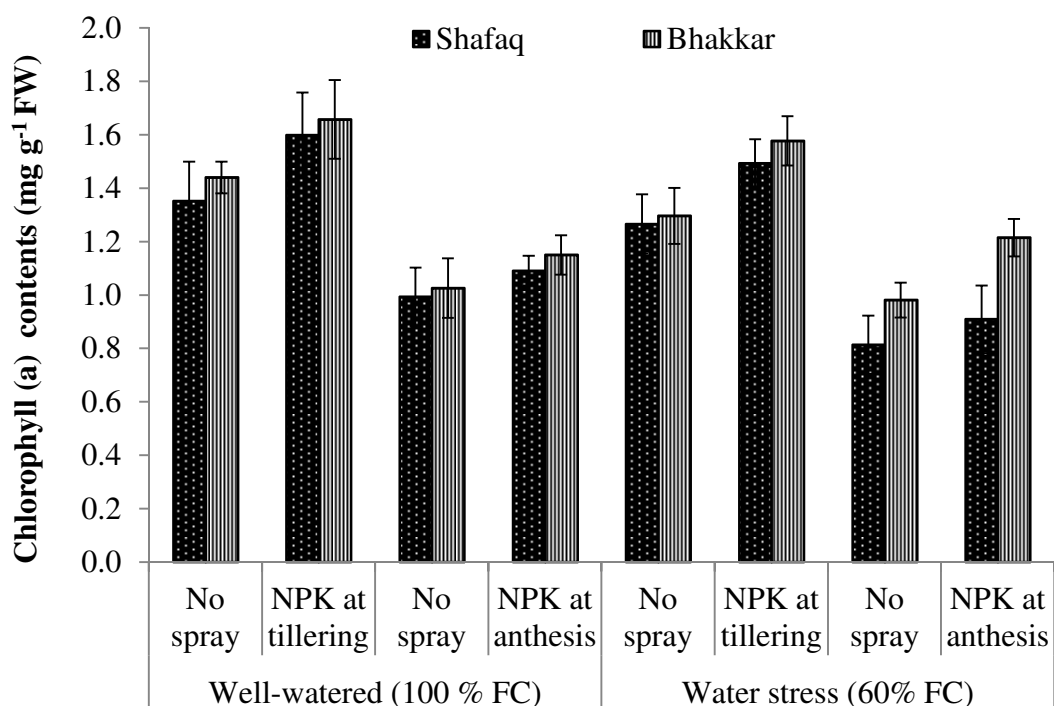


Fig. 4.28: Effect of supplemental foliar NPK application on chlorophyll (a) contents (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

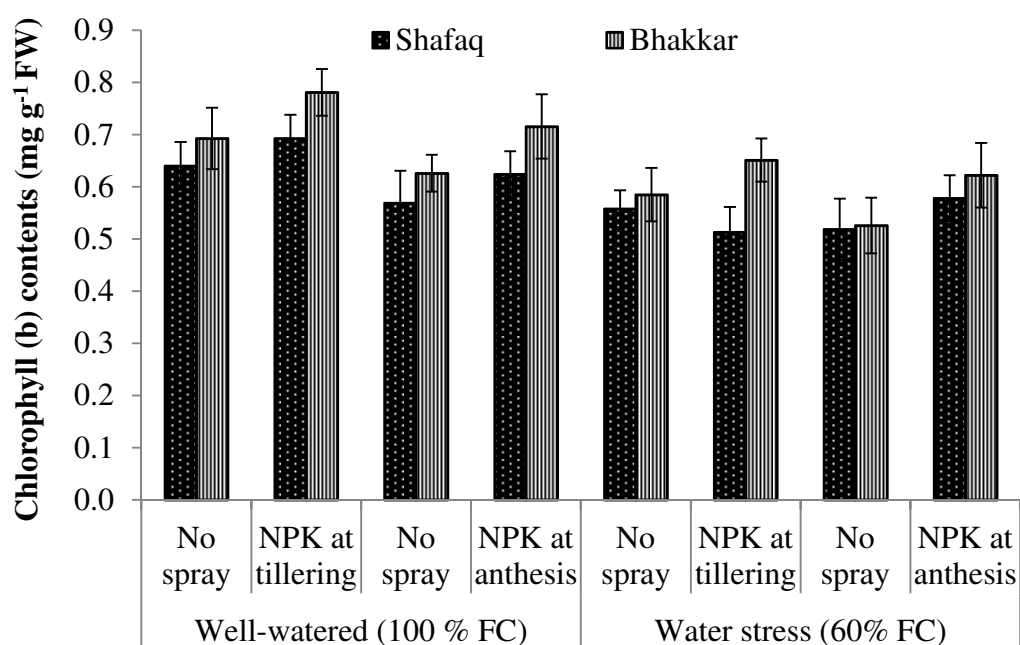


Fig. 4.29: Effect of supplemental foliar NPK application on chlorophyll (b) contents (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

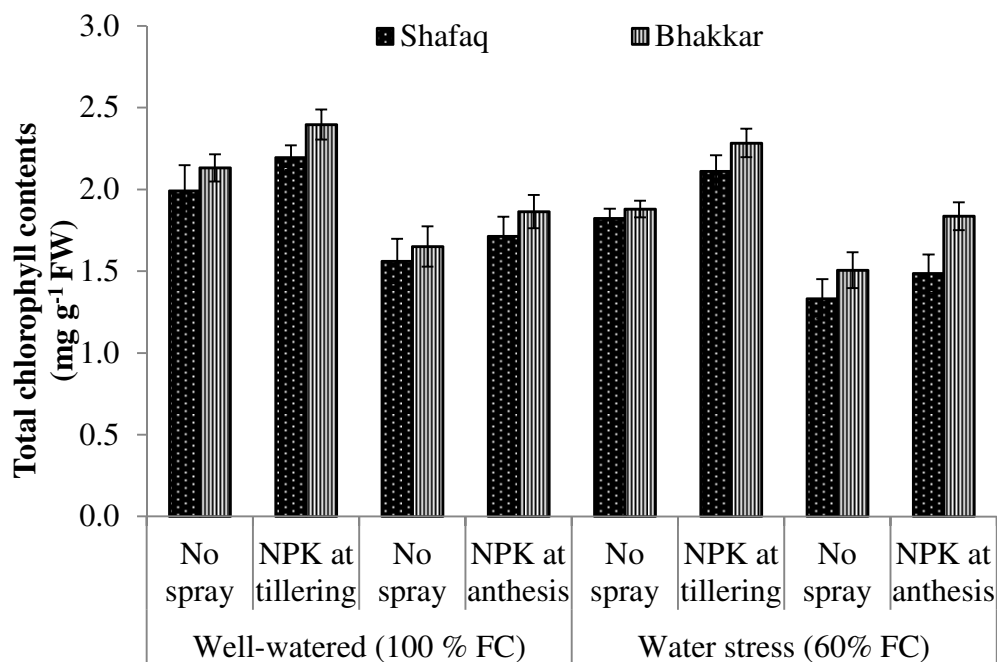


Fig. 4.30: Effect of supplemental foliar NPK application on total chlorophyll contents (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

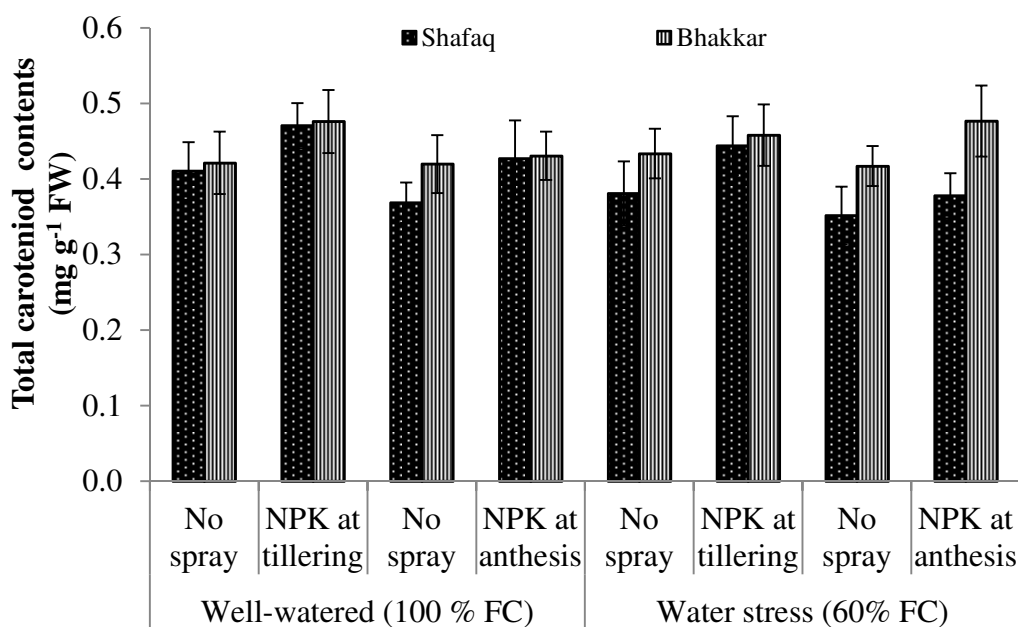


Fig. 4.31: Effect of supplemental foliar NPK application on total carotenoid contents (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

4.4.12. Leaf Total Soluble Sugars

Leaf total soluble sugars significantly increased under water limited conditions (Table 4.5). Drought stress increased total soluble sugars 17% and gave significantly higher values ($3.67 \text{ mg g}^{-1} \text{ FW}$) as compared to ($3.06 \text{ mg g}^{-1} \text{ FW}$) normal supply of water (Appendix 4.23). The comparison between growth stages indicated that accumulation of total soluble sugars at anthesis ($4.22 \text{ mg g}^{-1} \text{ FW}$) were more than at tillering stage ($2.51 \text{ mg g}^{-1} \text{ FW}$). Wheat genotypes exhibited highly significant ($P \leq 0.001$) difference for leaf total soluble sugars (Table 4.5). The highest value ($3.71 \text{ mg g}^{-1} \text{ FW}$) was recorded in drought tolerant Bhakkar-02 and minimum value ($3.02 \text{ mg g}^{-1} \text{ FW}$) was recorded in drought sensitive Shafaq-06 (Appendix 4.24). The foliar spray of NPK significantly ($P \leq 0.001$) increased for leaf total soluble sugars. Highest value ($3.85 \text{ mg g}^{-1} \text{ FW}$) was recorded for foliar applied NPK and lowest value ($2.87 \text{ mg g}^{-1} \text{ FW}$) was obtained in no spray wheat plants. Three-way interaction was also statistically significant for leaf total soluble sugars. Interaction between GXTXS was statistically significant ($P \leq 0.05$). Maximum total soluble sugars were observed in Bhakkar-02 with foliar NPK spray at anthesis stage and minimum was observed in Shafaq-06 with no spray at tillering stage (Fig. 4.32). All other interactions among different factors were non-significant for leaf total soluble sugars contents (Table 4.5).

4.4.13. Leaf Total Soluble Proteins

The plants exposed to water stress showed a significant ($P < 0.001$) decrease in total soluble proteins (Table 4.5). Drought stress reduced total soluble proteins by 5% with respect to normal conditions. The maximum value ($11.46 \text{ mg g}^{-1} \text{ FW}$) for total chlorophyll contents were recorded in normally grown plants while minimum ($8.10 \text{ mg g}^{-1} \text{ FW}$) was recorded in water stressed plants. The decrease in total soluble proteins was more pronounced at tillering ($9.53 \text{ mg g}^{-1} \text{ FW}$) than anthesis ($10.02 \text{ mg g}^{-1} \text{ FW}$) stage (Appendix 4.26). Analysis of variance for the data regarding leaf total soluble proteins showed highly significant ($P \leq 0.001$) difference between genotypes (Table 4.5). The plants of Bhakkar-02 maintained significantly higher leaf total soluble proteins ($10.18 \text{ mg g}^{-1} \text{ FW}$) than Shafaq-06 ($9.38 \text{ mg g}^{-1} \text{ FW}$), (Appendix 4.26).

The foliar spray of NPK significantly ($P \leq 0.001$) increased total soluble proteins in wheat plants and gave maximum value ($10.20 \text{ mg g}^{-1} \text{ FW}$) for this variable whereas no

NPK supply resulted in minimum total soluble proteins ($9.36 \text{ mg g}^{-1} \text{ FW}$) (Appendix 4.25). Interaction between GXS revealed highly significant ($P \leq 0.01$) difference. Highest value was observed in Bhakkar-02 at anthesis and lowest value was observed in Shafaq-06 at tillering (Fig.4.33). Interaction between GXW was highly ($P \leq 0.01$) significant for leaf total soluble proteins. Maximum total soluble proteins were recorded in Bhakkar-02 in normal plants and minimum was recorded in Shafaq-06 in water stressed plants (Fig.4.33). Interaction between SXW was statistically significant. Highest value for total soluble proteins was recorded at anthesis in normal plants and lowest value was recorded at tillering in both normal and water stressed plants (Fig.4.33). All other interactions among different factors were non-significant for leaf total soluble protein contents (Table 4.5).

4.4.14. Total Free Amino Acids

Leaf total free amino acids significantly increased under water limited conditions (Table 4.5). Drought stress increased total free amino acids (20%) and gave significantly higher values ($22.70 \text{ mg g}^{-1} \text{ FW}$) as compared to ($18.26 \text{ mg g}^{-1} \text{ FW}$) normal supply of water (Appendix 4.27). The comparison between growth stages indicated that accumulation of free amino acids at anthesis ($22.23 \text{ mg g}^{-1} \text{ FW}$) were more than at tillering stage ($18.72 \text{ mg g}^{-1} \text{ FW}$).

Analysis of variance for the data for total free amino acids revealed highly significant ($P \leq 0.01$) difference for genotypes. The highest value ($21.14 \text{ mg g}^{-1} \text{ FW}$) was recorded in drought tolerant Bhakkar-02 and minimum ($19.81 \text{ mg g}^{-1} \text{ FW}$) was recorded in drought sensitive Shafaq-06 (Appendix 4.28) The foliar spray of NPK significantly ($P \leq 0.001$) increased total free amino acids in wheat plants and gave maximum value ($21.16 \text{ mg g}^{-1} \text{ FW}$) for this variable whereas no NPK supply resulted in minimum total free amino acids ($19.79 \text{ mg g}^{-1} \text{ FW}$) (Appendix 4.27).

Interaction between SXW revealed highly significant ($P \leq 0.001$) difference for this variable. The highest value for total free amino acid was recorded at anthesis in water stressed plants and lowest value was observed at tillering in normal plants (Fig.4.34). All other interactions among different factors were non-significant for leaf total free amino acid contents (Table 4.5).

Table 4.5: Analysis of variance tables for total soluble sugars, total soluble proteins and total free amino acids (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions with foliar applied nutrient NPK.

SOV	Total soluble Sugars (mg g⁻¹ FW)	Total soluble proteins (mg g⁻¹ FW)	Total free amino acids (mg g⁻¹ FW)
Genotypes (G)	***	***	**
Treatment (T)	***	***	*
Stage (S)	***	***	***
Water levels (W)	***	***	***
GXT	NS	NS	NS
GXS	NS	**	NS
GXW	*	**	NS
TXS	NS	NS	NS
TXW	**	NS	NS
SXW	**	***	***
GXTXS	*	NS	NS
GXTXW	NS	NS	NS
GXSXW	NS	NS	NS
TXSXW	NS	NS	NS
GXTXSXW	NS	NS	NS
CV ^a	11.52	4.93	8.75

*, **, *** = Significant at 0.05, 0.01 and 0.001 level

NS = Non significant respectively, ^a coefficient of variation.

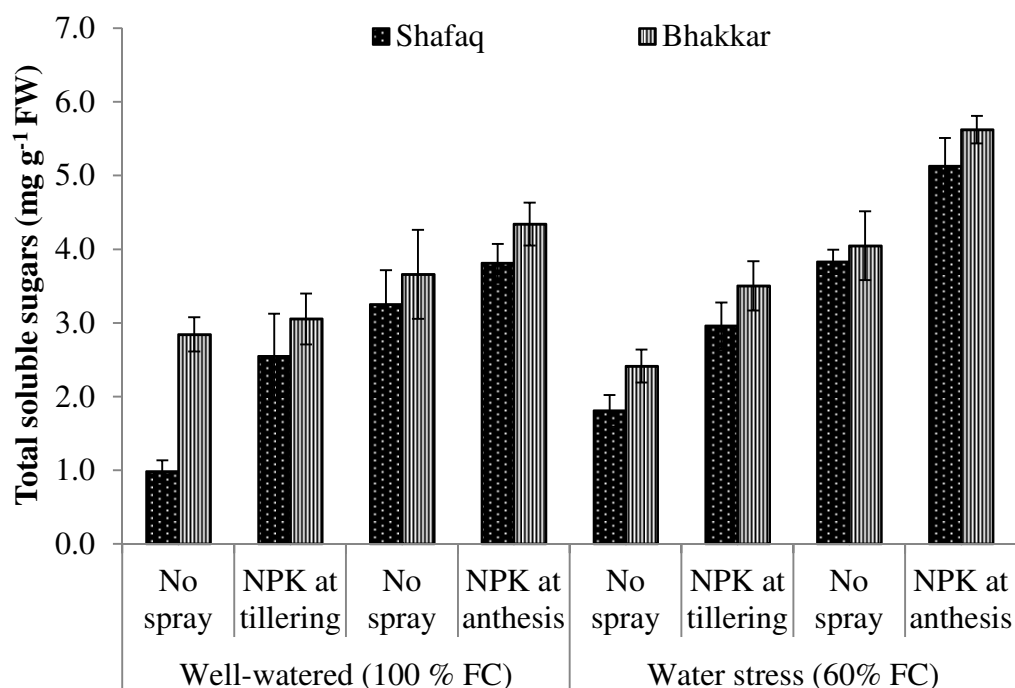


Fig. 4.32: Effect of supplemental foliar NPK application on total soluble sugars (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

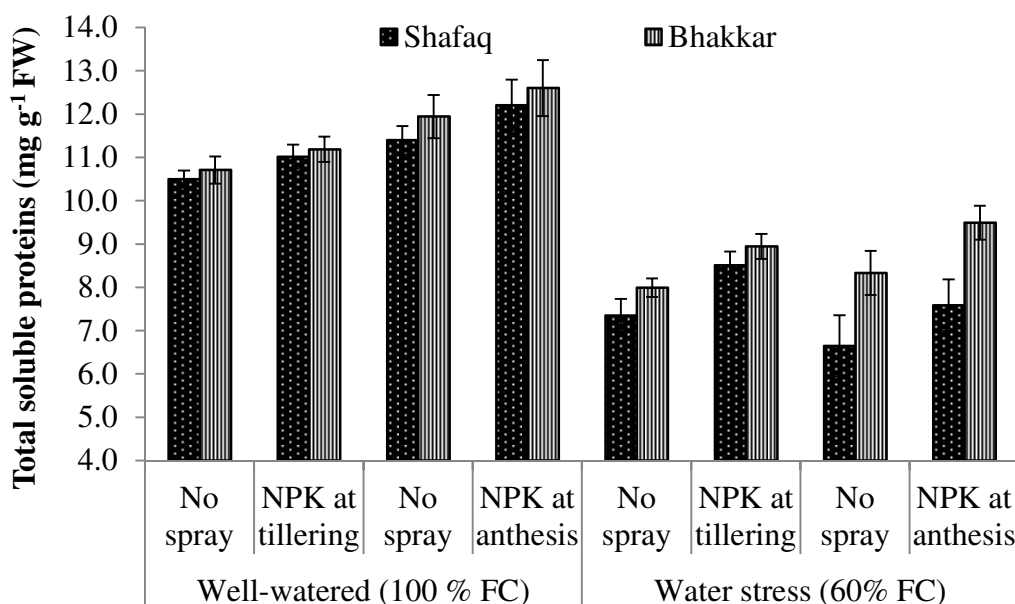


Fig. 4.33: Effect of supplemental foliar NPK application on total soluble proteins (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

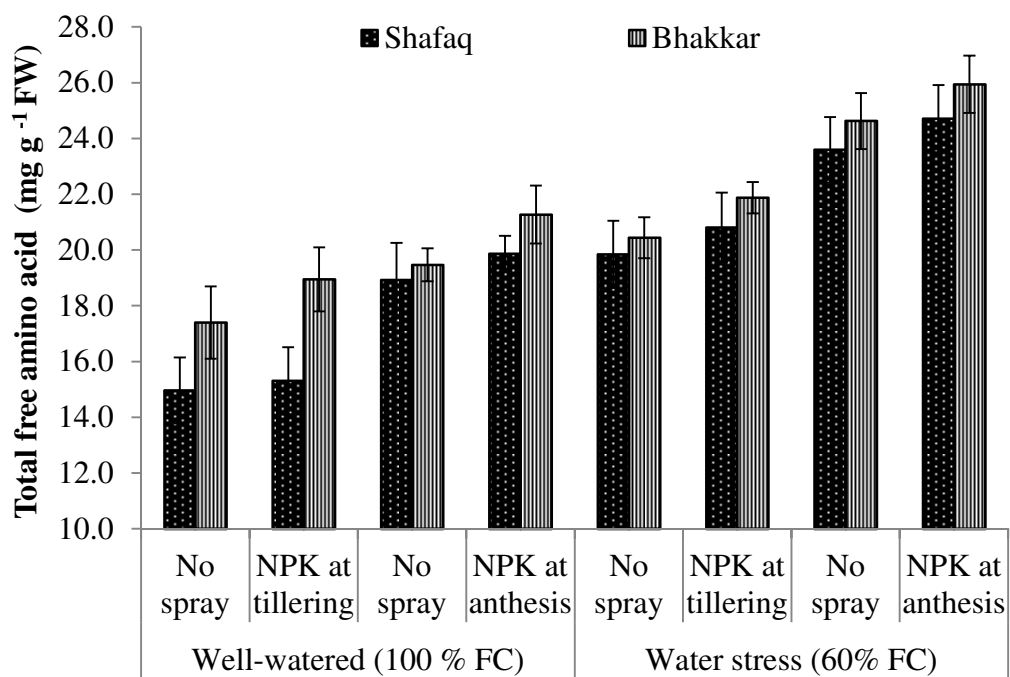


Fig. 4.34: Effect of supplemental foliar NPK application on total free amino acid (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

4.4.15. Leaf Nitrate Reductase Activity

Leaf nitrate reductase activity significantly decreased under water stress conditions (Table 4.6). Drought stress decreased nitrate reductase activity 17% and gave significantly lower values ($3.44 \mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) as compared to ($4.12 \mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) normal supply of water (Appendix 4.29). The comparison between growth stages exhibited non-significant for nitrate reductase activity (Appendix 4.30).

Wheat genotypes also showed non-significant difference for leaf for nitrate reductase activity. However, foliar spray of NPK significantly ($P \leq 0.001$) increased nitrate reductase activity. The highest value ($4.47 \mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) was recorded for foliar applied NPK and lowest value ($3.09 \mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) was obtained in no spray wheat plants.

Analysis of variance for the data for leaf nitrate reductase activity revealed highly significant interaction between GXTXSXW. Maximum value ($5.93 \mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) was recorded in Bhakkar-02 with foliar NPK treatment at anthesis stage in normal (100% FC) plants and minimum was recorded in Bhakkar-02 ($2.09 \mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) at tillering in water stressed (60% FC) plants (Fig. 4.35). Leaf nitrate reductase activity increased with foliar application of NPK under both normal and water stress conditions.

4.4.16. Leaf Nitrite Reductase Activity

Activity of leaf nitrite reductase significantly decreased under water stress conditions (Table 4.6). Drought stress decreased nitrite reductase activity 26% and gave significantly lower values ($3.01 \mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) as compared to ($4.08 \mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) normal supply of water (Appendix 4.31). The comparison between growth stages exhibited non-significant for nitrite reductase activity (Appendix 4.32).

Wheat genotypes also showed highly significant ($P \leq 0.001$) difference for leaf nitrite reductase activity. Maximum value ($3.91 \mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) was recorded in drought tolerant Bhakkar-02 and minimum ($3.17 \mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) was recorded in drought sensitive Shafaq-06.

The foliar spray of NPK significantly ($P \leq 0.001$) increased nitrite reductase activity. The highest value ($4.29 \mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) was recorded for foliar applied NPK and lowest value ($2.80 \mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) was recorded in no spray wheat plants.

Three-way interaction was also statistically significant for nitrite reductase activity. Interaction between TXSXW was statistically significant ($P \leq 0.05$) for this variable. Maximum value for leaf nitrite reductase was observed in foliar applied NPK spray at tillering stage in normal plant and minimum value was observed in no spray plants at anthesis stage in water stressed plants (Fig. 4.36).

All other interactions among different factors were non-significant for nitrite reductase activity (Table 4.6).

4.4.17. Leaf Proline Content

The plants exposed to water stress showed a significant ($P < 0.001$) increased in leaf proline contents (Table 4.6). Drought stress increased leaf proline contents by 27% with respect to normal conditions (Appendix 4.33). The increase in leaf proline contents was more pronounced at anthesis (2.78 mmol proline g⁻¹ FW) than tillering (1.08 mmol proline g⁻¹ FW) stage (Fig. 4.37). Highly significant ($P < 0.001$) effect of NPK application was observed on leaf proline contents of wheat plants. The plants foliarly sprayed with NPK gave maximum value (2.24 mmol proline g⁻¹ FW) than control (no NPK spray) (1.62 mmol proline g⁻¹ FW) plants. Wheat genotype Bhakkar-02 exhibited higher (15%) proline contents than Shafaq-06 (Appendix 4.34).

Interaction between GX SXW was statistically significant ($P \leq 0.05$). Maximum value for leaf proline contents was noted in foliar NPK spray at anthesis stage in water stressed (60% FC) plants and minimum value for proline contents was noted in no spray at tillering stage in normal (100% FC) plants.

All other interactions among different factors were non-significant for leaf proline content (Table 4.6).

Table 4.6: Analysis of variance tables for nitrate reductase, nitrite reductase ($\mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) and Proline ($\text{mmol proline g}^{-1} \text{ FW}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions with foliar applied nutrient NPK.

SOV	Nitrate Reductase ($\mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$)	Nitrite Reductase ($\mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$)	Proline ($\text{mmol proline g}^{-1} \text{ FW}$)
Genotypes (G)	NS	***	*
Treatment (T)	***	***	***
Stage (S)	NS	NS	***
Water levels (W)	***	***	***
GXT	***	NS	NS
GXS	*	NS	NS
GXW	**	NS	NS
TXS	NS	NS	*
TXW	NS	NS	**
SXW	NS	NS	NS
GXTXS	NS	*	NS
GXTXW	NS	NS	NS
GXSXW	NS	NS	NS
TXSXW	NS	***	*
GXTXSXW	**	NS	NS
CV ^a	11.13	11.39	22.0

*, **, *** = Significant at 0.05, 0.01 and 0.001 level

NS = Non significant respectively, ^a coefficient of variation.

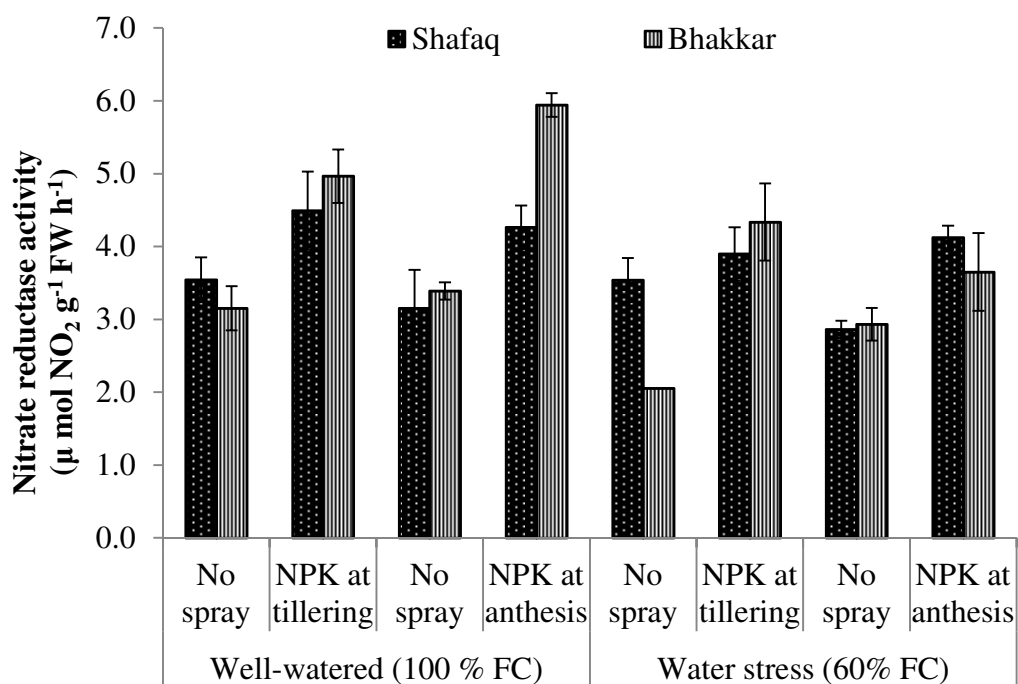


Fig. 4.35: Effect of supplemental foliar NPK application on nitrate reductase ($\mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

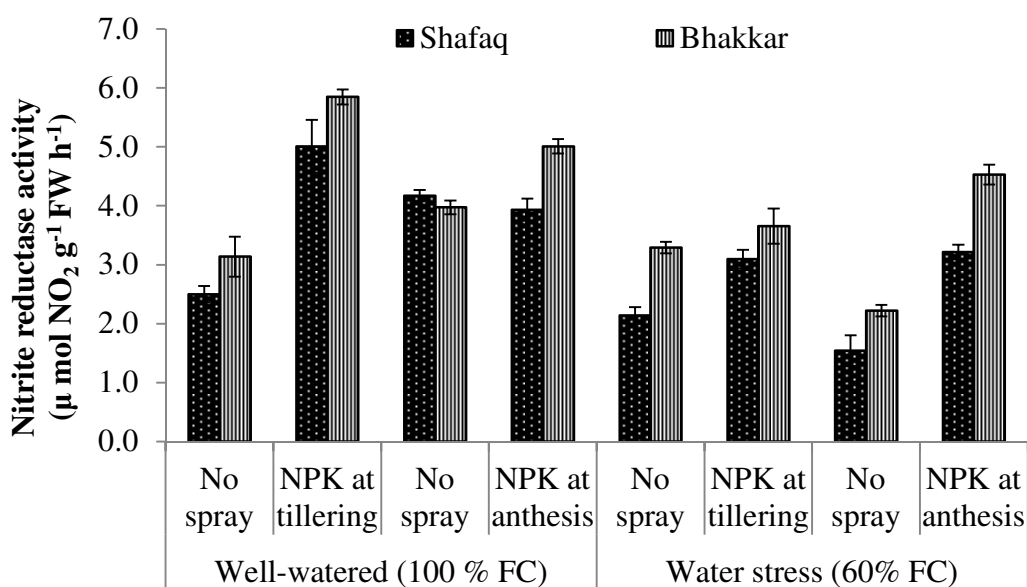


Fig. 4.36: Effect of supplemental foliar NPK application on nitrite reductase ($\mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

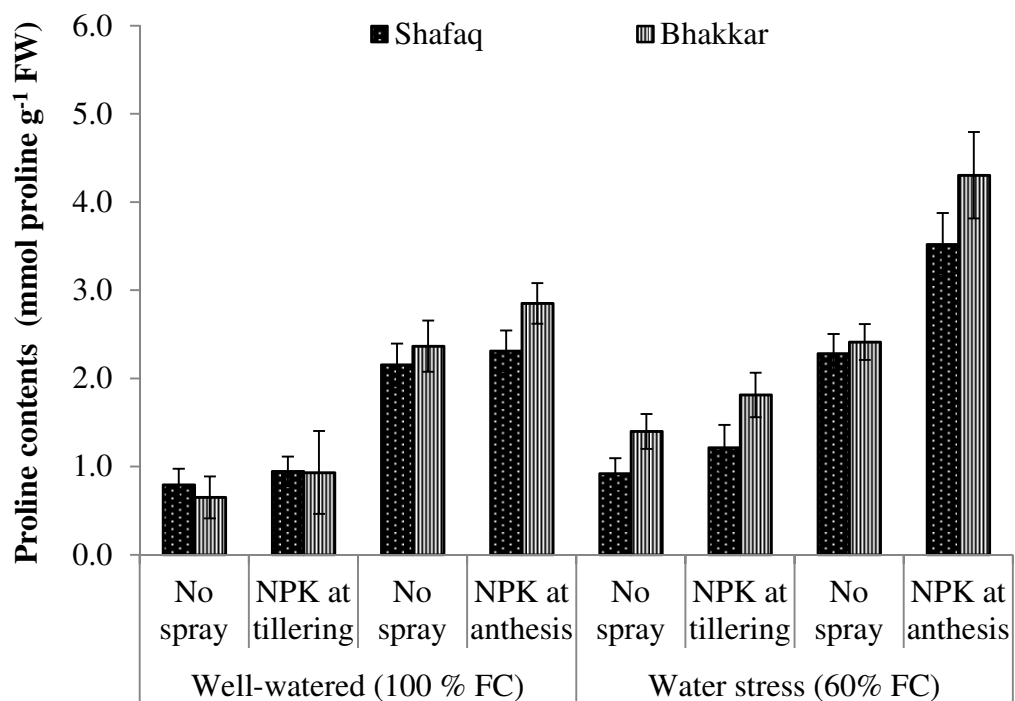


Fig. 4.37: Effect of supplemental foliar NPK application on proline contents (mmol proline g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

4.4.18. Ascorbate Peroxidase Activity

Leaf ascorbate peroxidase activity significantly increased in water stress conditions (Table 4.7). Drought stress increased leaf ascorbate peroxidase activity by 40% with respect to normal conditions. The maximum ascorbate peroxidase activity (1.90 ABA digested g^{-1} FW h^{-1}) was recorded in water stressed (60 % FC) plants and minimum (1.14 ABA digested g^{-1} FW h^{-1}) was recorded in normal (100 % FC) wheat plants (Appendix 4.35). The increase in leaf ascorbate peroxidase activity was more pronounced at anthesis (2.06 ABA digested g^{-1} FW h^{-1}) than tillering (0.98 ABA digested g^{-1} FW h^{-1}) stage (Appendix 4.36). Wheat genotypes also showed highly significant ($P \leq 0.01$) difference for leaf ascorbate peroxidase activity. Maximum value (1.65 ABA digested g^{-1} FW h^{-1}) was recorded in drought tolerant Bhakkar-02 and minimum (1.39 ABA digested g^{-1} FW h^{-1}) was recorded in drought sensitive Shafaq-06 (Appendix 4.36). Foliar application of NPK spray increased ascorbate peroxidase activity 15% and gave significantly higher values (1.64 ABA digested g^{-1} FW h^{-1}) as compared to (1.40 ABA digested g^{-1} FW h^{-1}) no spray wheat plants.

Interaction between SXW was highly significant ($P \leq 0.001$) different for ascorbate peroxidase activity. Maximum leaf ascorbate peroxidase activity was recorded at anthesis in water stress (60% FC) plants, while minimum was recorded at tillering in water stress (60% FC) plants (Fig. 4.38). All other interactions were non-significant for ascorbate peroxidase activity (Table 4.7).

4.4.19. Catalase Activity

Leaf catalase activity significantly increased in water stress conditions. Drought stress increased leaf catalase activity by 13% with respect to normal conditions. Maximum value (249.58 units min^{-1} g^{-1} FW) for leaf catalase activity was recorded in water stress (60% FC) plants and lowest value (218.25 units min^{-1} g^{-1} FW) was recorded in normal (100% FC) plants (Appendix 4.37). The increase in leaf catalase activity was more pronounced at anthesis (322.58 units min^{-1} g^{-1} FW) than tillering (145.25 units min^{-1} g^{-1} FW) stage (Appendix 4.38). Wheat genotypes also showed highly significant ($P \leq 0.05$) difference for leaf catalase activity (Table 4.7). Maximum value (249.38 units min^{-1} g^{-1} FW) was recorded in drought tolerant Bhakkar-02 and minimum (218.46 units min^{-1} g^{-1} FW) was recorded in drought sensitive Shafaq-06 (Appendix 4.38). Foliar application of NPK spray increased catalase activity 14% and gave significantly higher values (251.25

units $\text{min}^{-1} \text{g}^{-1} \text{FW}$) as compared to (216.58 units $\text{min}^{-1} \text{g}^{-1} \text{FW}$) no spray wheat plants (Appendix 4.37).

Interaction between GXTXW showed significant ($P \leq 0.01$) differences (Table 4.7). Highest value for leaf catalase activity was recorded in Bhakkar-02 with foliar NPK spray in water stress (60% FC) plants and lowest value was recorded in Shafaq-06 with no spray in normal (100% FC) plants. Interaction between TXSXW showed highly significant ($P \leq 0.01$) differences for this variable. Maximum value for leaf catalase activity was recorded in foliar NPK at anthesis in water stress (60% FC) plants and lowest value was recorded in no spray at tillering stage in normal (100% FC) plants (Fig. 4.39). All other interactions were non-significant for catalase activity (Table 4.7).

4.4.20. Peroxidase Activity

Leaf peroxidase activity significantly increased under water limited conditions (Table 4.7). Drought stress increased peroxidase activity (3%) and gave significantly higher values (773.24 unit $\text{min}^{-1} \text{g}^{-1} \text{FW}$) as compared to (753.86 unit $\text{min}^{-1} \text{g}^{-1} \text{FW}$) normal supply of water (Appendix 4.39). The comparison between growth stages indicated that accumulation of peroxidase activity at anthesis (774.88 unit $\text{min}^{-1} \text{g}^{-1} \text{FW}$) were more than at tillering stage (752.22 unit $\text{min}^{-1} \text{g}^{-1} \text{FW}$).

Analysis of variance for the data for peroxidase showed highly significant ($P \leq 0.001$) difference for genotypes (Table 4.7). Maximum value (771.32 unit $\text{min}^{-1} \text{g}^{-1} \text{FW}$) was recorded in drought tolerant Bhakkar-02 and minimum (755.78 unit $\text{min}^{-1} \text{g}^{-1} \text{FW}$) was recorded in drought sensitive Shafaq-06 (Appendix 4.40). Highly significant ($P \leq 0.001$) differences were observed among treatments for peroxidase (Table 4.7). The highest value (777.29 unit $\text{min}^{-1} \text{g}^{-1} \text{FW}$) was recorded for foliar applied NPK spray and lowest value (749.81 unit $\text{min}^{-1} \text{g}^{-1} \text{FW}$) was recorded in no spray treatment. Two-way interaction was statistically significant for peroxidase activity. Interaction between TXW revealed significant ($P \leq 0.05$) difference. The highest value peroxidase activity was recorded with foliar NPK spray in water stress (60% FC) plants whereas, lowest value was observed with no spray in normal (100% FC) wheat plants (Fig. 4.40).

All interactions were non-significant for peroxidase activity (Table 4.7).

Table 4.7: Analysis of variance table for ascorbate peroxidase (ABA digested g^{-1} FW h^{-1}) catalase ($\text{units min}^{-1} \text{g}^{-1}$ FW) and peroxidase ($\text{units min}^{-1} \text{g}^{-1}$ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions with foliar applied nutrient NPK.

SOV	Ascorbate Peroxidase (ABA digested g^{-1} FW h^{-1})	Catalase ($\text{units min}^{-1} \text{g}^{-1}$ FW)	Peroxidase ($\text{units min}^{-1} \text{g}^{-1}$ FW)
Genotypes (G)	**	***	***
Treatment (T)	**	***	***
Stage (S)	***	***	***
Water levels (W)	***	***	***
GXT	NS	*	NS
GXS	NS	NS	NS
GXW	NS	*	NS
TXS	NS	***	NS
TXW	NS	**	*
SXW	***	***	NS
GXTXS	NS	NS	NS
GXTXW	NS	**	NS
GXSXW	NS	NS	NS
TXSXW	NS	**	NS
GXTXSXW	NS	NS	NS
CV ^a	20.86	6.38	1.58

*, **, *** = Significant at 0.05, 0.01 and 0.001 level

NS = Non significant respectively, ^a coefficient of variation.

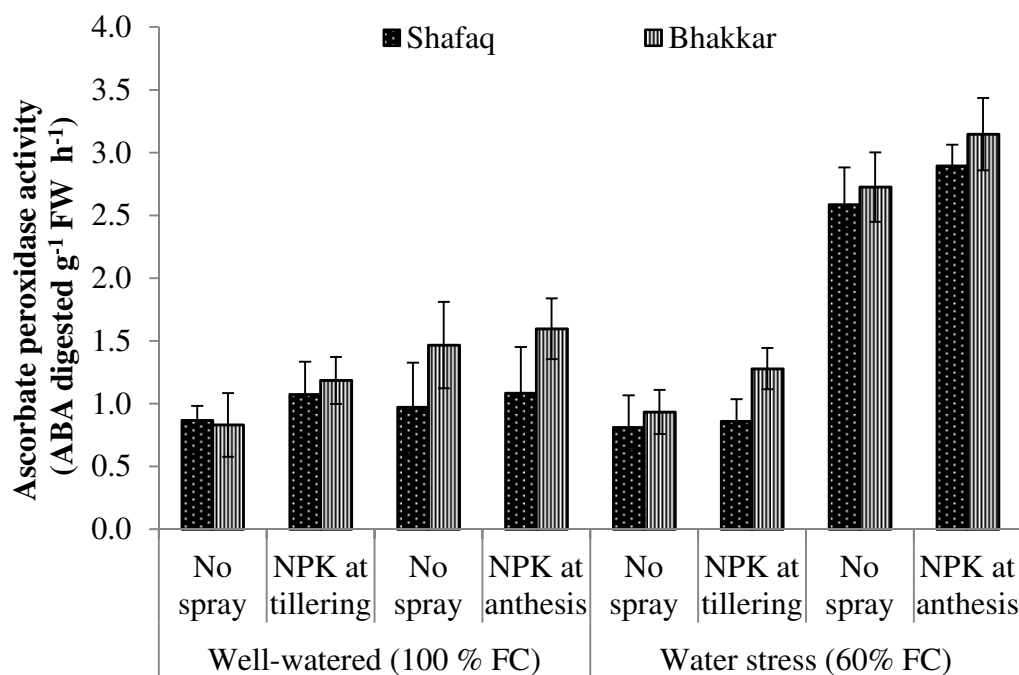


Fig. 4.38: Effect of supplemental foliar NPK application on ascorbate peroxidase activity (ABA digested g⁻¹ FW h⁻¹) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (means ± S.E).

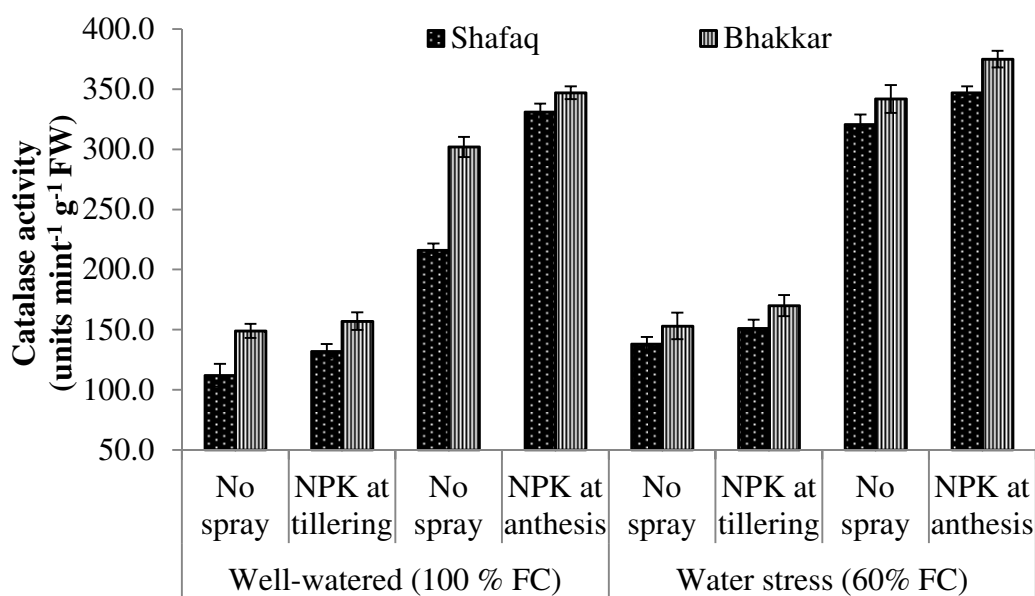


Fig. 4.39: Effect of supplemental foliar NPK application on catalase activity (units min⁻¹ g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values ± S.E).

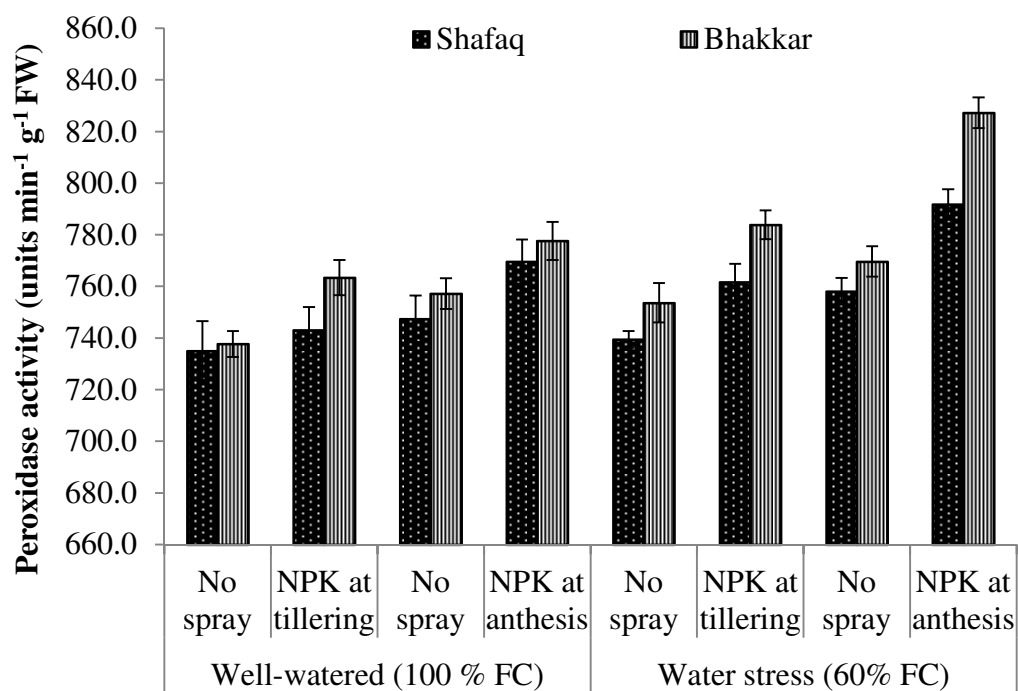


Fig. 4.40: Effect of supplemental foliar NPK application on peroxidase activity (units min⁻¹ g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (means $\pm$ S.E).

4.4.21. Leaf Nitrogen Contents (N)

Wheat plants exhibited significantly higher ($P \leq 0.001$) leaf N content under water stress (60% FC) than normal conditions (100% FC) (Table 4.8). The exposure to drought stress increased leaf nitrogen content by 7% as compared to normal supply of water. The maximum (23.78 mg g⁻¹DW) leaf N contents was recorded in water stressed plants and minimum was recorded in (23.78 mg g⁻¹DW) in normal plants (Appendix 4.41).

Analysis of variance for the data regarding leaf N content showed highly significant ($P \leq 0.001$) difference between genotypes (Table 4.8). The plants of Bhakkar-02 maintained significantly higher leaf N content (26.33 mg g⁻¹ DW) than Shafaq-06 (23.13 mg g⁻¹ DW), (Appendix 4.42). The foliar spray of NPK significantly ($P \leq 0.001$) increased leaf N content in wheat plants and gave maximum value (25.71 mg g⁻¹DW) for this variable whereas no NPK application resulted in minimum leaf N content (23.75 mg g⁻¹ DW). A highly significant difference was also observed between growth stages for this variable. The plants exhibited higher leaf N content at tillering (32.87 mg g⁻¹ DW) than anthesis (16.87 mg g⁻¹ DW) stage (Appendix 4.42).

Two-way interaction was statistically significant for nitrogen content. Interaction between GXW revealed significant ($P \leq 0.01$) difference (Table 4.8). The highest value for nitrogen content was observed in Bhakkar-02 in water stress (60% FC) plants and lowest value was observed in Shafaq-06 in normal (100% FC) plant (Fig. 4.41).

All other interactions were non-significant for leaf N contents (Table 4.8).

4.4.22. Leaf Phosphorous Contents (P)

The data regarding leaf P contents revealed highly significant effect of drought stress on this variable. The limited water supply reduced leaf P contents (22%) as compared to normal conditions. The maximum P contents were recorded in normal (100% FC) plant and minimum was recorded in water stress (60% FC) plants (Appendix 4.43). The plants maintained significantly higher (15%) leaf P contents at anthesis (4.17 mg g⁻¹DW) than tillering (3.55 mg g⁻¹DW) stage (Appendix 4.44).

The genotypes also varied significantly for this variable (Table.4.8). Wheat genotype Bhakkar-02 (4.51 mg g⁻¹ DW) exhibited higher leaf P contents than Shafaq-06 (3.20 mg g⁻¹ DW), (Appendix 4.44). The foliar spray of NPK was highly effective in

improving leaf P. The plants foliarly applied with NPK gave significantly higher value ($4.2 \text{ mg g}^{-1} \text{ DW}$) than those with no spray ($3.51 \text{ mg g}^{-1} \text{ DW}$), (Appendix 4.43).

Three-way interaction was also showed significant differences for P contents. Interaction between GXTXW revealed significant ($P \leq 0.05$) differences (Table.4.8). Highest value for P contents was recorded in Bhakkar-02 in foliar NPK spray in normal (100% FC) plants and lowest value was recorded in Shafaq-06 with no spray in water stress (60% FC) plants (Fig. 4.42). All other interactions among different factors were non-significant for leaf P contents (Table.4.8).

4.4.23. Leaf Potassium Contents (K)

Wheat plants exhibited significantly higher ($P \leq 0.001$) leaf K content under water stress (60% FC) than normal conditions (100% FC) (Table 4.8). The exposure to drought stress increased leaf K^+ content by 19% and gave higher ($37.90 \text{ mg g}^{-1} \text{ DW}$) as compared to normal supply of water ($30.67 \text{ mg g}^{-1} \text{ DW}$) (Appendix 4.45).

Analysis of variance for the data regarding leaf K content showed highly significant ($P \leq 0.001$) difference between genotypes. The plants of Bhakkar-02 maintained significantly higher leaf K^+ content ($36.84 \text{ mg g}^{-1} \text{ DW}$) than Shafaq-06 ($31.73 \text{ mg g}^{-1} \text{ DW}$). The foliar spray of NPK significantly ($P \leq 0.001$) increased leaf K content in wheat plants and gave maximum value ($36.93 \text{ mg g}^{-1} \text{ DW}$) for this variable whereas no NPK supply resulted in minimum leaf K^+ content ($31.64 \text{ mg g}^{-1} \text{ DW}$). A highly significant difference was also observed between growth stages for this variable. The plants exhibited higher leaf K content at anthesis ($35.98 \text{ mg g}^{-1} \text{ DW}$) than tillering ($32.58 \text{ mg g}^{-1} \text{ DW}$) stage (Appendix 4.46).

Two-way interaction was statistically significant for leaf K content (Fig. 4.41). Interaction between SXW revealed significant ($P \leq 0.05$) difference (Table 4.8). Highest value for leaf K content was recorded at anthesis stage under water stress (60% FC) and lowest value was recorded at tillering under well-watered (100% FC) plants (Fig. 4.43).

All other interactions were non-significant for leaf K^+ (Table 4.8).

Table 4.8: Analysis of variance tables for nitrogen, phosphorous and potassium (mg g⁻¹ DW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions with foliar applied nutrient NPK.

SOV	Nitrogen (mg g⁻¹ DW)	Phosphorous (mg g⁻¹ DW)	Potassium (mg g⁻¹ DW)
Genotypes (G)	***	***	***
Treatment (T)	**	***	***
Stage (S)	***	***	***
Water levels (W)	**	***	***
GXT	NS	*	NS
GXS	NS	**	NS
GXW	**	NS	NS
TXS	NS	NS	NS
TXW	NS	**	NS
SXW	NS	*	*
GXTXS	NS	NS	NS
GXTXW	NS	*	NS
GXSXW	NS	NS	NS
TXSXW	NS	NS	NS
GXTXSXW	NS	NS	NS
CV ^a	8.70	10.14	8.56

*, **, *** = Significant at 0.05, 0.01 and 0.001 level

NS = Non significant respectively, ^a coefficient of variation.

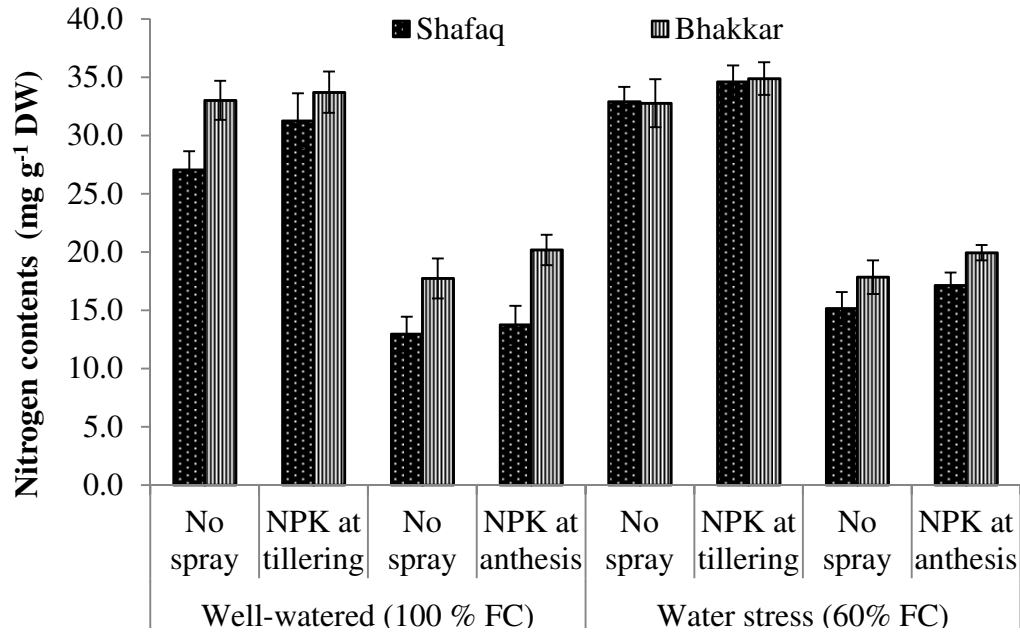


Fig. 4.41: Effect of supplemental foliar NPK application on nitrogen contents (mg g⁻¹ DW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

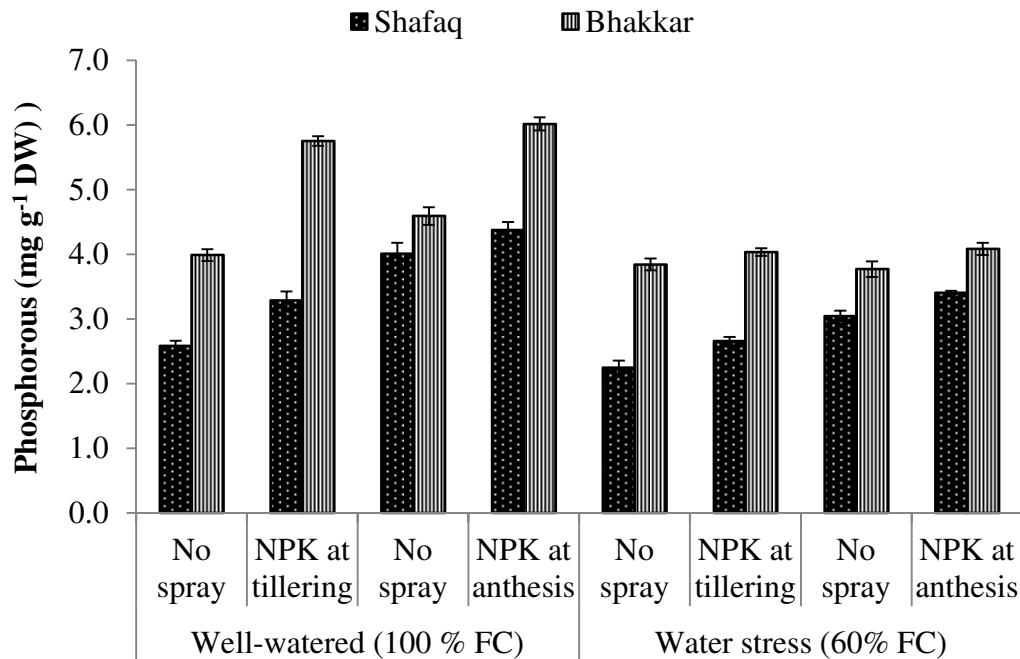


Fig. 4.42: Effect of supplemental foliar NPK application on phosphorous conc. (mg g⁻¹ DW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

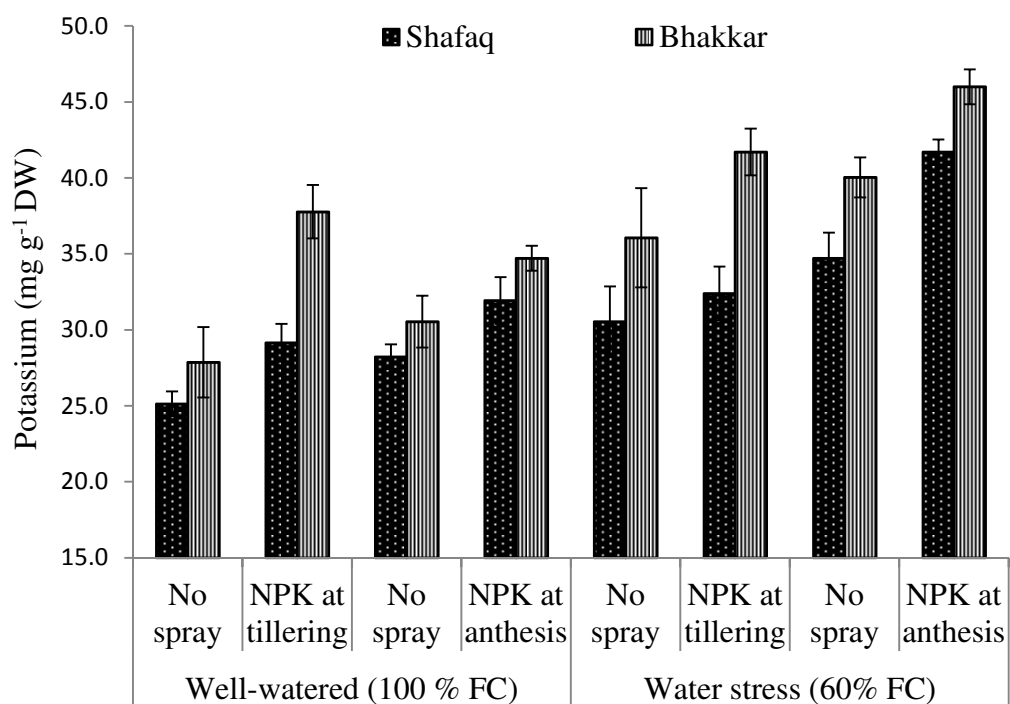


Fig. 4.43: Effect of supplemental foliar NPK application on potassium conc. (mg g⁻¹ DW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions (mean values $\pm$ S.E).

4.5. Field Experiments

4.5.1. Number of Tillers

The number of tillers were significantly ($P<0.01$) reduced by drought stress in wheat genotypes during the year 2011-12 and 2012-13 (Table 4.9a, b). The decrease in number of tillers was more prominent in water stressed plants at early stages of growth i.e. tillering stage. During 2011-12, the water stress at tillering stage reduced number of tillers by 11% as compared to normally irrigated plants (Appendix 4.47). Similar trend was observed in the year 2012-13 where plants exposed to drought stress at tillering stage showed little higher reduction (12%) for this variable as compare to normally irrigated plants. Wheat genotype Shafaq-06 produced only 2% higher number of tillers as compared to Bhakkar-02 in 2011-12, while in 2012-13 both genotypes showed non-significant difference (Table 4.9b).

Highly significant difference ($P<0.001$) for foliar NPK spray was observed for this variable. In 2011-12, plants foliarly sprayed with NPK at tillering stage increase number of tillers 7% (576.28) as compared to no spray plants (537.95) and were statistically at par with foliar applied NPK at anthesis stage (567.28) during 2011-12. During 2012-13, the plants foliarly sprayed with NPK produced 7% higher number of tillers (569.22) as compare to control plants (530.61), (Appendix 4.47).

The interaction among different factors GXWXT was significant during 2011-12 for this variable but non-significant during 2012-13 (Table 4.9a, b). Wheat genotype Shafaq-06 maintained maximum (614) number of tillers in foliarly applied NPK plants exposed to water stress at anthesis stage, which was statistically at par with Shafaq-06 (595.33) with foliar applied NPK spray at tillering in normally irrigated plants. The minimum (450) number of tillers was recorded in Shafaq-06 with no spray treatment in water stress at tillering stage (Figs. 4.44, 4.45).

4.5.2. Number of Fertile Tillers

Drought stress significantly decreased ($P<0.001$) the number of fertile tillers during both the years (Table 4.9a, b). In 2011-12, the water limited conditions at tillering and anthesis stage significantly decreased the number of fertile tillers by 18% and 6% respectively, while during the second year (2012-13), reduction was 19% and 5% respectively as compared to normally irrigated (control) plants (Appendix 4.48)

Wheat genotype Bhakkar-02 and Shafaq-06 were non-significant in both the years i.e. 2011-12 and 2012-13 respectively (Appendix 4.50) (Table 4.9a, b). The effect of foliar NPK spray treatment was also highly significant ($P<0.001$) for number of fertile tillers. During 2011-12 and 2012-13, foliar application of NPK spray at tillering increased the number of fertile tillers 10% (423.89), (418.28) as compare to no spray (380.0), (377.0) treatment (Appendix 4.48).

The interaction among different factors GXWXT was also significant during both years for this variable. During 2011-12, 2012-13, the maximum (467.33), (458.33) number of fertile tillers were recorded in Bhakkar-02 with foliar applied NPK spray at anthesis stage in normally irrigated plant which was statistically at par in Bhakkar-02 (453.33), (448.33) with foliar applied NPK spray at tillering in normally irrigated plants. The minimum (306.67), (299.0) number of fertile tillers were recorded in Shafaq-06 with no spray treatment in water stress at tillering stage (Fig. 4.46, 4.47).

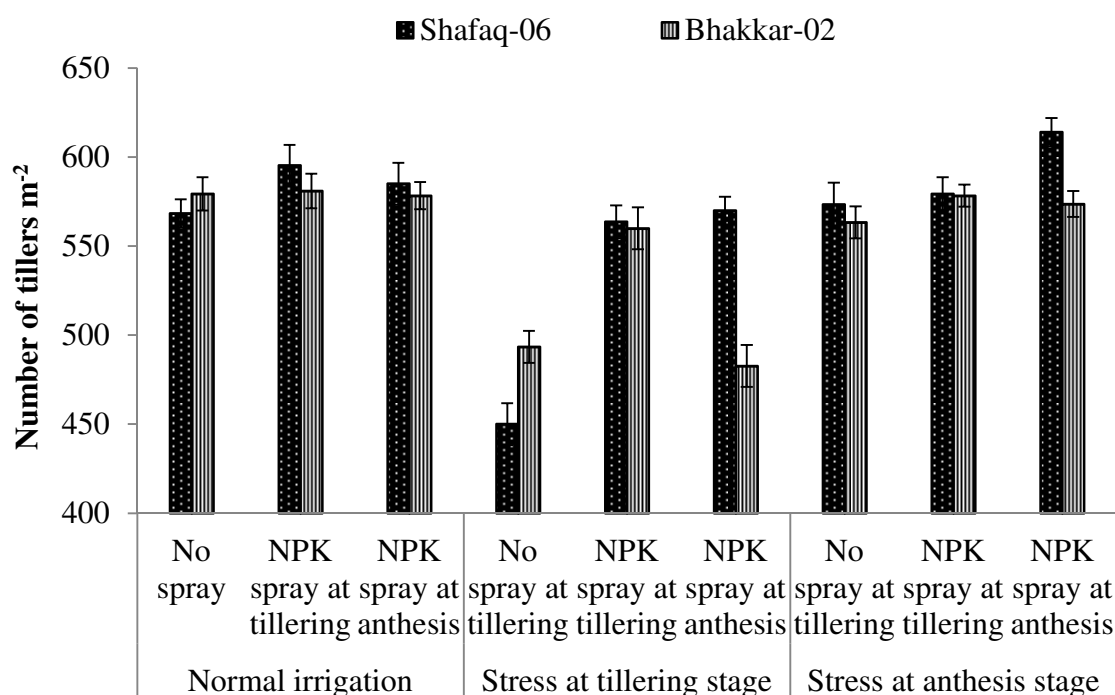


Fig. 4.44: Effect of supplemental foliar NPK application on number of tillers of two wheat genotypes under different water levels during 2011-12 (mean values $\pm$ S.E).

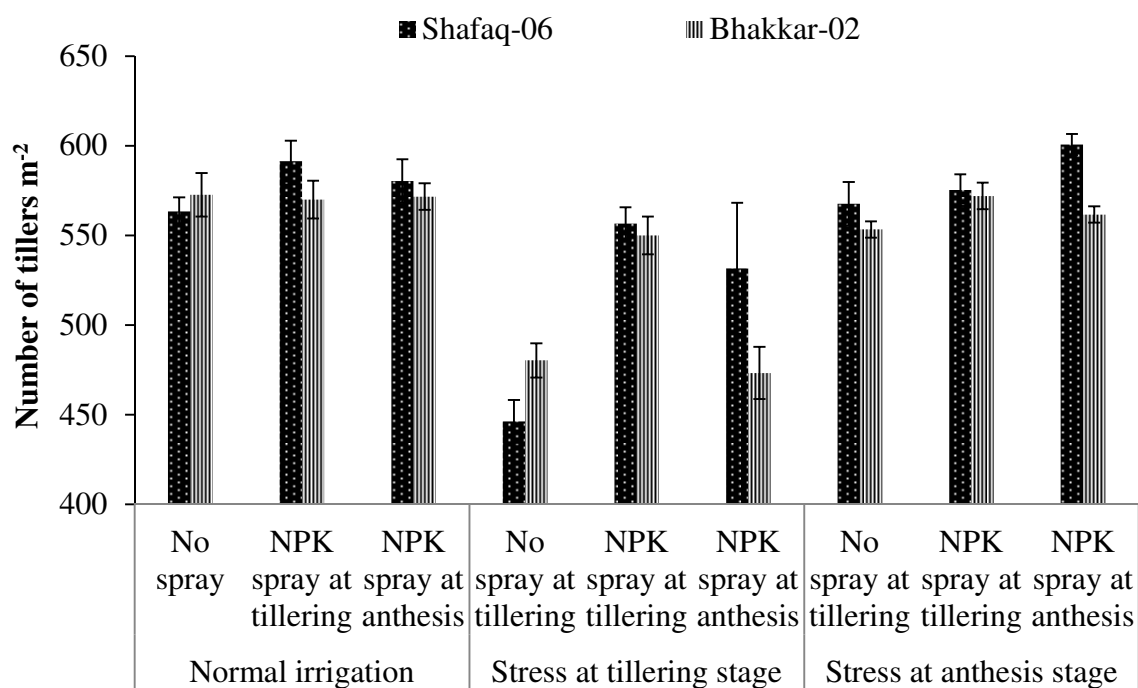


Fig. 4.45: Effect of supplemental foliar NPK application on number of tillers of two wheat genotypes under different water levels during 2012-13 (mean values $\pm$ S.E).

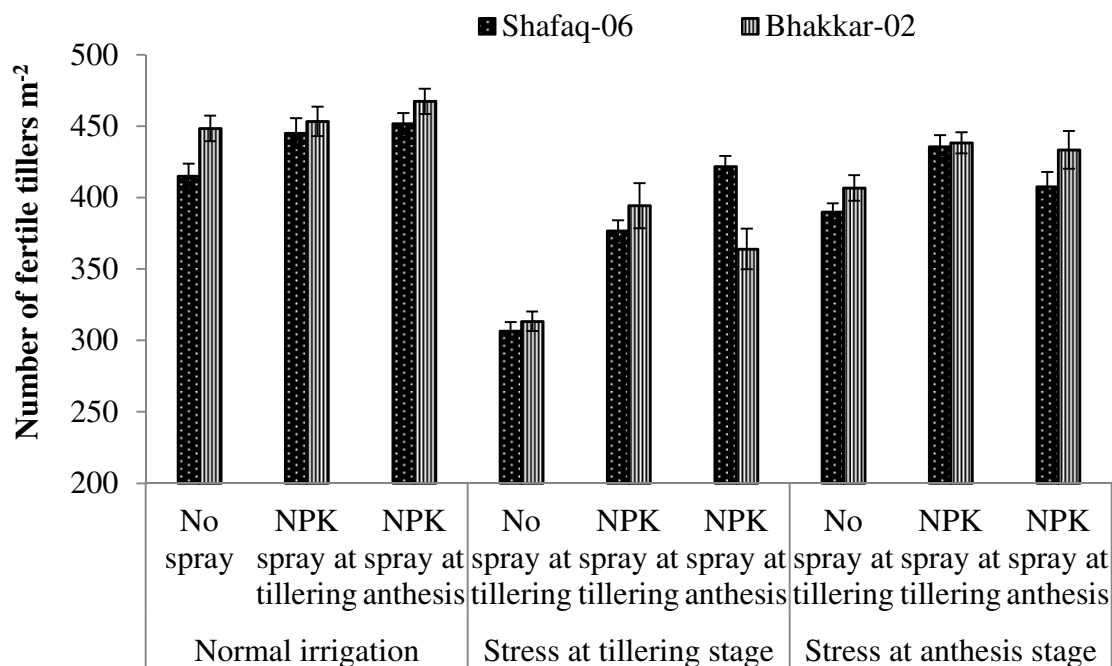


Fig. 4.46: Effect of supplemental foliar NPK application on number of fertile tillers of two wheat genotypes under different water levels during 2011-12 (mean values $\pm$ S.E).

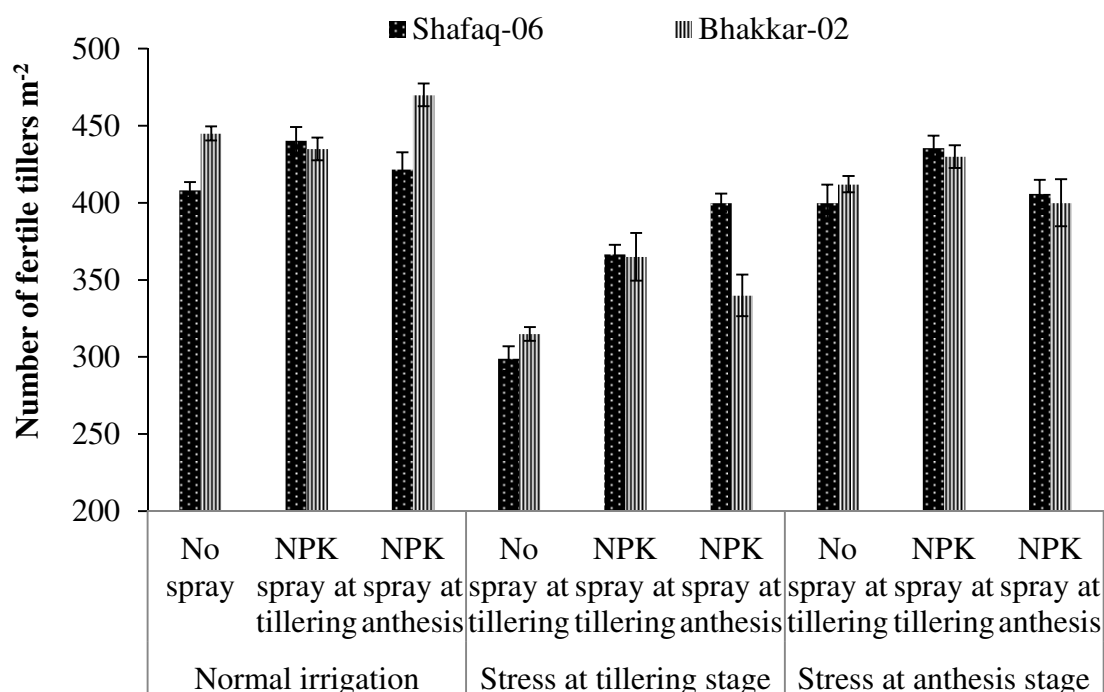


Fig. 4.47: Effect of supplemental foliar NPK application on number of fertile tillers of two wheat genotypes under different water levels during 2012-13 (mean values $\pm$ S.E).

4.5.3. Plant Height

The plant height was significantly ($P<0.01$) reduced by drought stress in wheat genotypes during the year 2011-12 and 2012-13 (Table 4.9a, b). The decrease in plant height was more pronounced in water stressed plants at early stage of growth i.e. tillering stage. During 2011-12, the water stress at tillering stage reduced plant height by 4% as compared to normally irrigated plants. Similar trend was observed in the year 2012-13 where plants exposed to drought stress at tillering stage showed little higher reduction (5%) for this variable as compare to normally irrigated plants (Appendix 4.49). Wheat genotype Bhakkar-02 increased only 2% and 1% more plant height as compared to Shafaq-06 in 2011-12 and 2012-13. Highly significant difference ($P<0.001$) for foliar NPK spray was observed for this variable. In 2011-12, 2012-13, plants foliarly sprayed with NPK at tillering stage plant height (95.13 cm, 95.14 cm) as compared to no spray (control) plants (91.26 cm, 91.28 cm), (Appendix 4.49).

The interaction GXT was significant in both the years (Table 4.9 a, b)., the plants of Bhakkar-02 foliarly applied with NPK at tillering stage increased plant height, while minimum value for plant height was recorded in Shafaq-6 in no spray (control) plants during 2011-12, 2012-13 (Figs. 4.48, 4.49). Interaction between GXW was highly significant in 2011-12 but non-significant during 2012-13. Wheat genotype Bhakkar-02 and Shafaq-06 maintained maximum plant height in normally irrigated plants, which was statistically at par with Bhakkar-02 exposed to water stress at anthesis stage. The minimum plant height was recorded in Shafaq-06 exposed to water stress at tillering stage during 2011-12 (Fig. 4.48). All other interactions were non-significant during both years (Table 4.9a, b).

4.5.4. Spike length

Drought stress significantly reduced ($P<0.001$) the spike length during both the years (Table 4.9a, b). In 2011-12, the water deficit conditions at tillering and anthesis stage significantly decreased it by 11% and 2% respectively (Fig. 4.50), while during the second year (2012-13), reduction was 12% and 1% respectively as compared to normally irrigated (control) plants (Appendix 4.50). Wheat genotype Bhakkar-02 maintained 8.9% and 9.12% more spike length than Shafaq-06 in both the years i.e. 2011-12 and 2012-13 respectively.

The effect of foliar applied NPK spray treatment was also highly significant ($P<0.001$) for spike length. During 2011-12 and 2012-13, foliar application of NPK spray at tillering increased the spike length 10% (11.71 cm), (11.63 cm) as compare to no spray (11.42 cm), (11.34 cm) treatment (Appendix 4.50).

The interaction WXT was also significant during both the years. During 2011-12, 2012-2013, the plants foliarly applied with NPK exposed to water stress at tillering and anthesis stage had maximum spike length, while minimum value for spike length was recorded in no spray (control) plants and foliarly applied NPK exposed to water stress at tillering stage (Figs. 4.50, 4.51). Interaction between GXW was highly significant in 2011-12 but non-significant during 2012-13. All other interactions were non-significant during both years (Table 4.9a, b).

Table 4.9a, b: Analysis of variance table for number of tillers, number of fertile tillers, plant height (cm) and spike length (cm) of two wheat genotypes in well-watered and water stress conditions with foliar applied nutrients NPK.

a.

SOV	Number of tillers	Number of fertile tillers	Plant height (cm)	Spike length (cm)
Genotypes (G)	*	NS	**	***
Water levels (W)	***	***	***	***
Treatments (T)	***	***	***	**
GXW	NS	*	***	**
GXT	***	NS	**	NS
WXT	***	***	NS	*
GXWXT	***	**	NS	NS
CV ^a	2.96	4.01	1.80	2.58

b.

SOV	Number of tillers	Number of fertile tillers	Plant height (cm)	Spike length (cm)
Genotypes (G)	NS	NS	**	***
Water levels (W)	***	***	***	***
Treatments (T)	***	***	***	**
GXW	NS	*	NS	NS
GXT	*	NS	***	NS
WXT	***	***	NS	**
GXWXT	NS	*	NS	NS
CV ^a	3.99	4.0	1.81	2.58

*, **, *** = Significant at 0.05, 0.01 and 0.001 level respectively

NS = Non significant; ^a coefficient of variation.

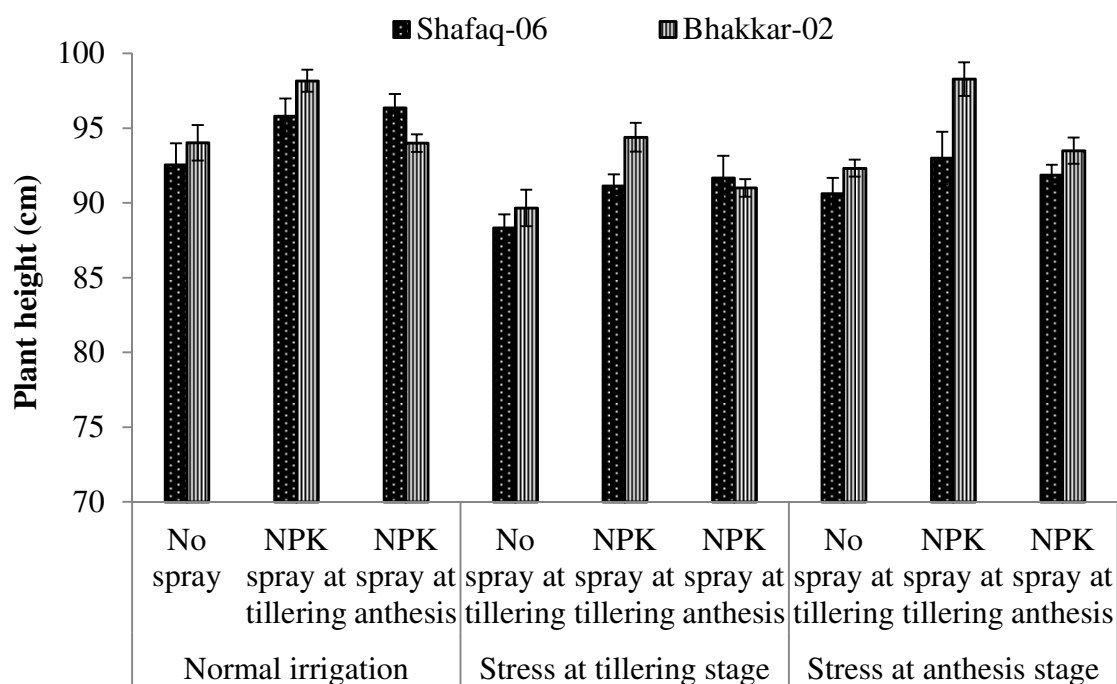


Fig. 4.48: Effect of supplemental foliar NPK application on plant height of two wheat genotypes under different water levels during 2011-12 (mean values $\pm$ S.E).

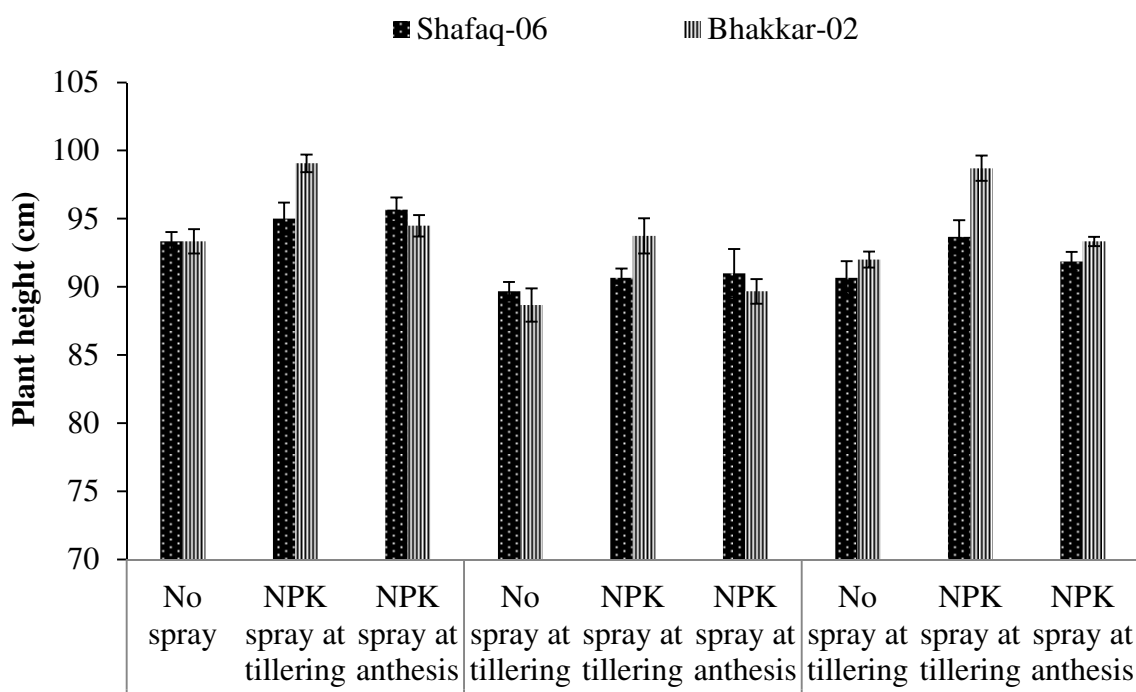


Fig. 4.49: Effect of supplemental foliar NPK application on plant height of two wheat genotypes under different water levels during 2012-13 (mean values $\pm$ S.E).

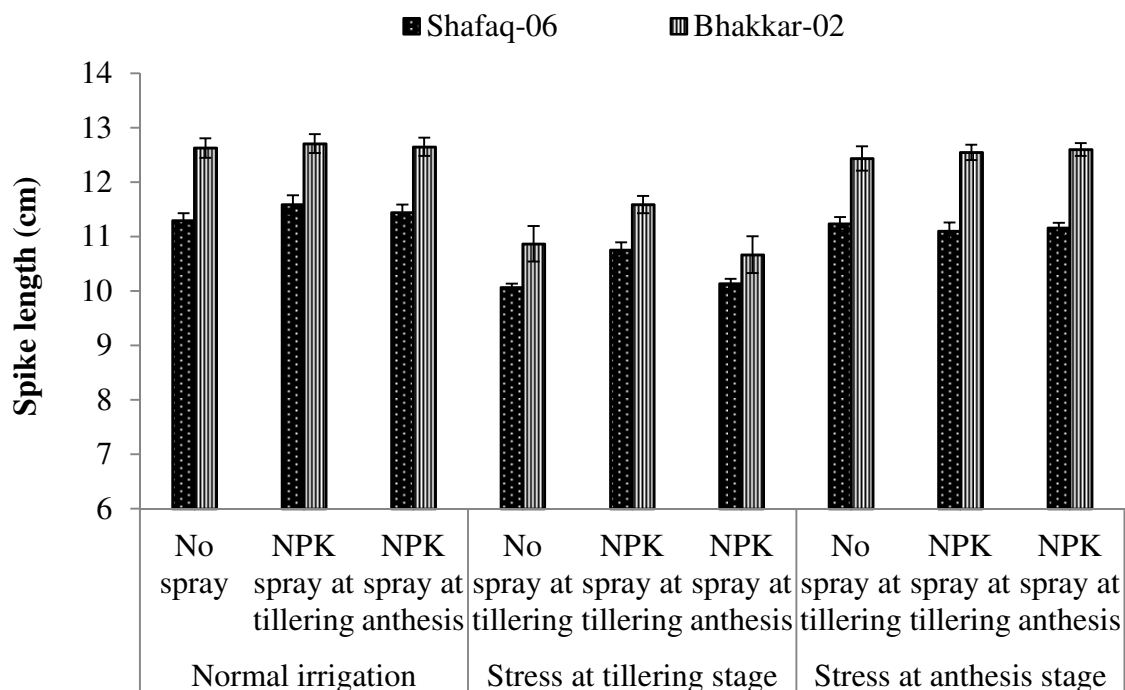


Fig. 4.50: Effect of supplemental foliar NPK application on spike length of two wheat genotypes under different water levels during 2011-12 (mean values $\pm$ S.E).

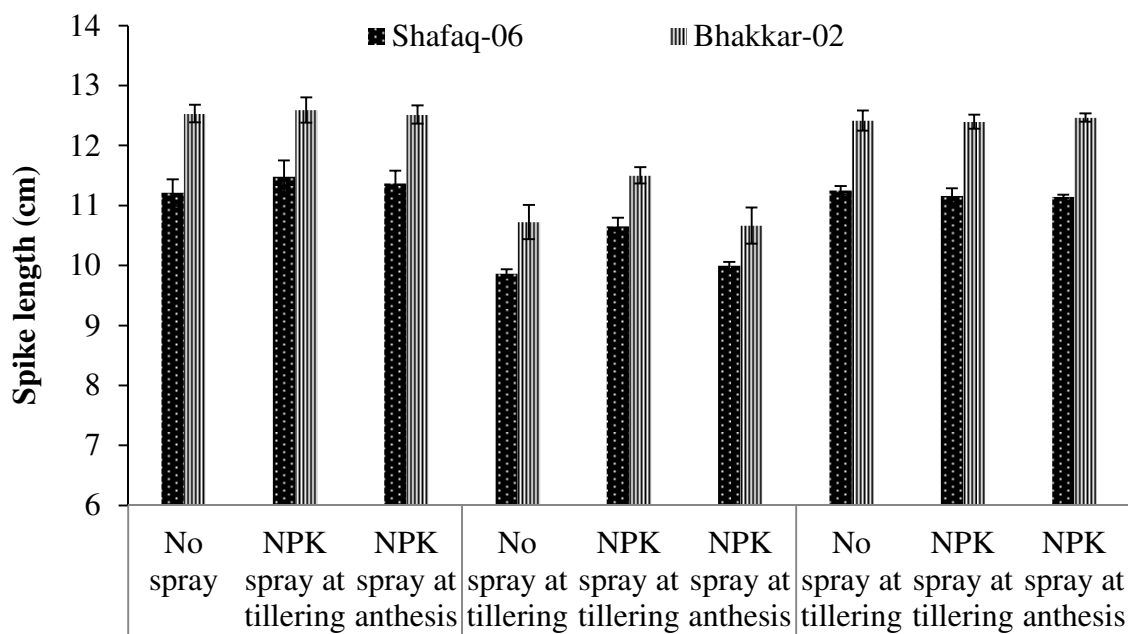


Fig. 4.51: Effect of supplemental foliar NPK application spike length of two wheat genotypes under different water levels during 2012-13 (mean values $\pm$ S.E).

4.5.5. Number of Spikelets Spike⁻¹

The number of spikelets spike⁻¹ was significantly ($P<0.01$) decreased by water stress in wheat genotypes during the year 2011-12 and 2012-13 (Table 4.10a, b). The decrease in number of spikelets spike⁻¹ was more pronounced in water stressed plants at early stage of growth i.e. tillering stage. During 2011-12, the water stress at tillering and anthesis stage reduced number of spikelets spike⁻¹ by 22% and 4% as compared to normally irrigated plants (Appendix 4.51). Similar trend was observed in the year 2012-13 where plants exposed to drought stress at tillering stage showed reduction of 21% and 2% respectively for this variable as compared to normally irrigated plants (Appendix 4.51), (Figs. 4.52, 4.53).

All other individual factors and their interactions were non-significant during both years for this variable (Table 4.10a, b).

4.5.6. Number of Grains Spike⁻¹

Drought stress significantly reduced ($P<0.001$) the number of grains spike⁻¹ during both the years (Table 4.10a, b). In 2011-12, the water deficit conditions at tillering and anthesis stage significantly decreased number of grains spike⁻¹ by 15% and 20% respectively (Fig. 4.54), while during the second year (2012-13), reduction was 15% and 18% respectively as compared to normally irrigated (control) plants (Appendix 4.52).

Wheat genotype Bhakkar-02 maintained 5% and 3% more number of grains spike⁻¹ than Shafaq-06 in both the years i.e. 2011-12 and 2012-13 respectively. The effect of foliar applied NPK spray treatment was also highly significant ($P<0.001$) for number of grains spike⁻¹. During 2011-12 and 2012-13, foliar application of NPK spray at anthesis increased the number of grains spike⁻¹ 19%, (43.94) and 18%, (45.0) as compared to no spray (35.77), (36.94) treatment (Appendix 4.52).

The interaction WXT was significant during both the years. During 2011-12, the plants foliarly applied with NPK under normal conditions at anthesis stage had maximum number of grains spike⁻¹ (46.17) which was statistically at par (44.67) with foliar applied NPK at tillering stage under normally irrigated plants and with foliar applied NPK spray (43.17) under water stress conditions at anthesis stage.

The minimum value (31.33) was recorded in no spray (control) plants under water stress conditions at anthesis stage which was statistically at par (33.0) with foliar applied NPK at tillering stage under water stressed plants at anthesis and with no spray (33.0) under water stress conditions at tillering stage, during 2011-12 (Fig. 4.54).

Similarly in 2012-13, the foliar NPK spray in normally irrigated plants at anthesis stage resulted in maximum (47.33) number of grains spike⁻¹, which was statistically at par (44.83) with foliar applied NPK at tillering stage under normally irrigated plants and while minimum value (32.0) was recorded in no spray (control) plants under water stress conditions at anthesis stage which was statistically at par (33.50) with no spray at tillering stage under water stress plants at tillering and with foliar NPK spray at tillering (34.67) under water stress conditions at anthesis stage (Fig. 4.55).

All other interactions were non-significant during both years (Table 4.10a, b).

4.5.7. Thousand Grain Weight

Water stress significantly decreased ($P<0.001$) the 1000-grain weight during 2011-12 and 2012-13 respectively (Table 4.10a, b). In 2011-12, the water deficit conditions at tillering and anthesis stage significantly decreased it by 22% and 30% respectively while during the second year (2012-13), reduction was 21% and 24% respectively as compared to control (Appendix 4.53).

Wheat genotype Bhakkar-02 maintained 6% higher 1000-grain weight than Shafaq-06 in 2012-13 but both genotypes were non-significant for 1000-grain weight in 2011-12 respectively (Fig. 4.56, 4.57).

The effect of foliar NPK spray treatments was also significant ($P<0.01$) for this variable. The foliar NPK spray at anthesis stage gave the maximum value (46.23 g), (47.11 g) and minimum value (38.52 g), (39.56 g) was recorded in no spray treatment during 2011-12 and 2012-13 respectively (Appendix 4.53). All other interactions were non-significant during both years (Table 4.10a, b).

4.5.8. Biological Yield

The biological yield was significantly ($P<0.001$) reduced by drought stress in both wheat genotypes during the year 2011-12 and 2012-13 (Table 4.10a, b). The decreased in biological yield was more prominent in water stressed plants at early stages of growth i.e. tillering stage.

During 2011-12 and 2012-13, the drought stress at tillering and anthesis stage decreased biological yield by 9% and 3% as compared to normally irrigated plants (Appendix 4.54). Wheat genotype Bhakkar-02 produced only 3% higher biological yield as compared to Shafaq-06 in 2011-12 and 2012 -13 (Figs. 4.58, 4.59).

Highly significant difference for foliar NPK spray was observed for this variable. The plants foliarly sprayed with NPK at tillering stage increased biological yield 5% and 9%, (11.43 Mg ha^{-1}), (11.45 Mg ha^{-1}) and were statistically at par with foliar applied NPK at anthesis stage (11.23 Mg ha^{-1}), (11.24 Mg ha^{-1}) as compared to no spray (control) plants (10.90 Mg ha^{-1}), (10.89 Mg ha^{-1}) during 2011-12, and 2012-13 (Appendix 4.54).

All interactions were non-significant for biological yield during both years (Table 4.10a, b).

Table 4.10a, b: Analysis of variance table for spikelets spike⁻¹, number of grains spike⁻¹, thousand grain weight (g) and biological yield (Mg ha⁻¹) of two wheat genotypes in well-watered and water stress conditions with foliar applied nutrients NPK.

a.

SOV	Number of spikelets spike⁻¹	Number of grains spike⁻¹	Thousand grain weight (g)	Biological yield (Mg ha⁻¹)
Genotypes (G)	NS	*	NS	*
Water levels (W)	***	***	***	***
Treatments (T)	NS	***	***	***
GXW	NS	NS	NS	NS
GXT	NS	NS	NS	NS
WXT	NS	***	NS	NS
GXWXT	NS	NS	NS	NS
CV ^a	8.96	6.72	5.16	4.70

b.

SOV	Number of spikelets spike⁻¹	Number of grains spike⁻¹	Thousand grain weight (g)	Biological yield (Mg ha⁻¹)
Genotypes (G)	NS	*	***	*
Water levels (W)	***	***	***	***
Treatments (T)	NS	***	***	**
GXW	NS	NS	NS	NS
GXT	NS	NS	NS	NS
WXT	NS	**	NS	NS
GXWXT	NS	NS	NS	NS
CV ^a	8.60	6.07	4.75	4.46

*, **, *** = Significant at 0.05, 0.01 and 0.001 level respectively

NS = Non significant; ^a coefficient of variation.

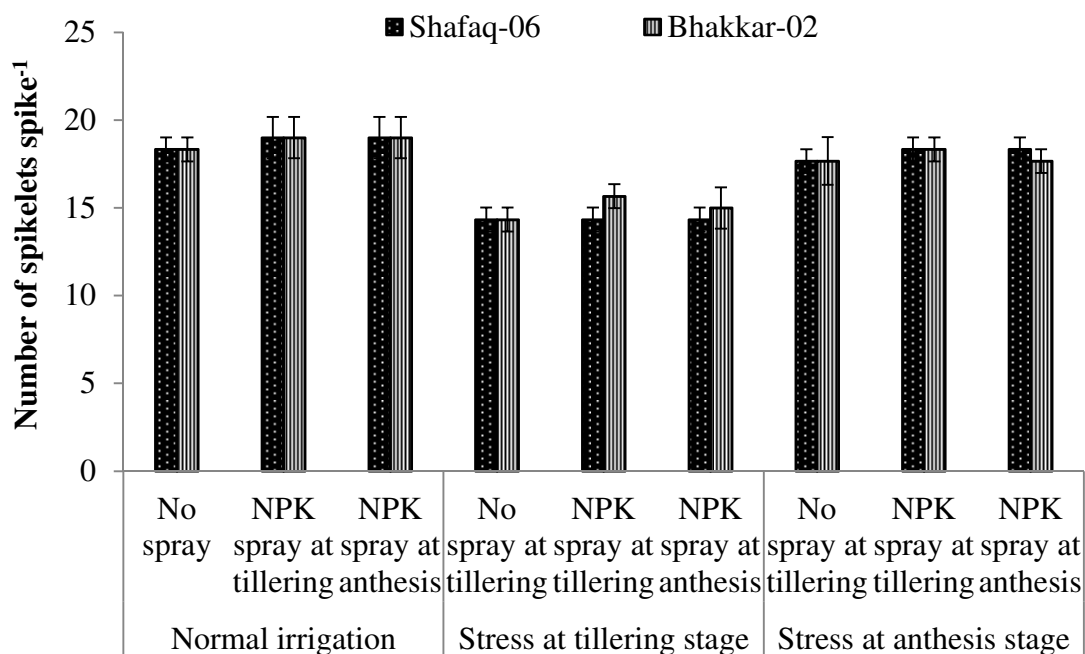


Fig. 4.52: Effect of supplemental foliar NPK application on number of spikelets spike⁻¹ of two wheat genotypes under different water levels during 2011-12 (mean values $\pm$ S.E).

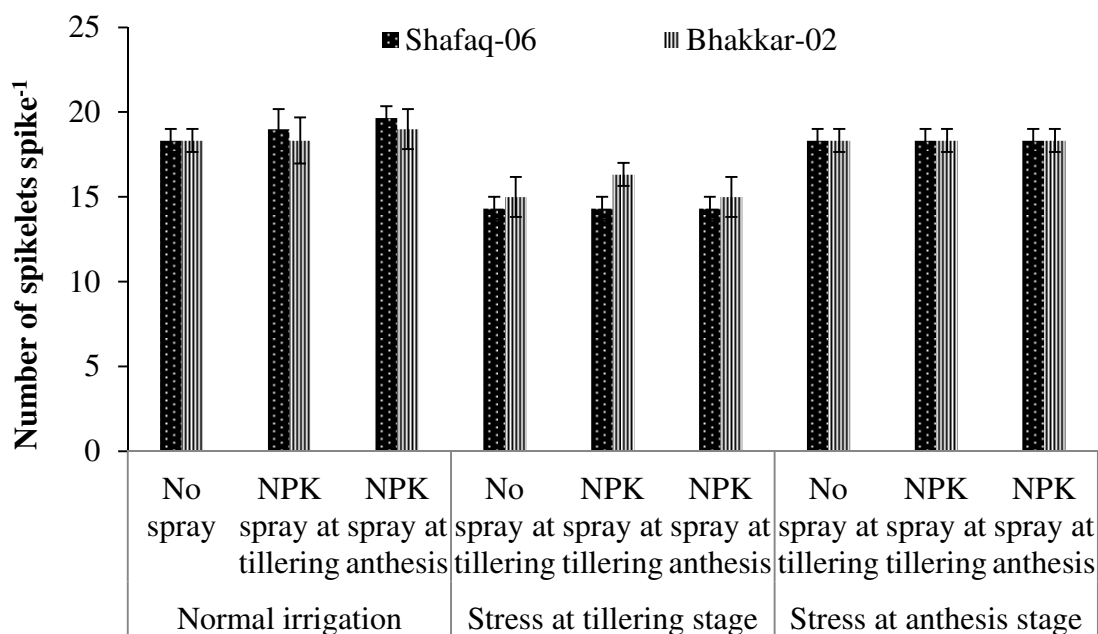


Fig. 4.53: Effect of supplemental foliar NPK application on number of spikelets spike⁻¹ of two wheat genotypes under different water levels during 2012-13 (mean values $\pm$ S.E).

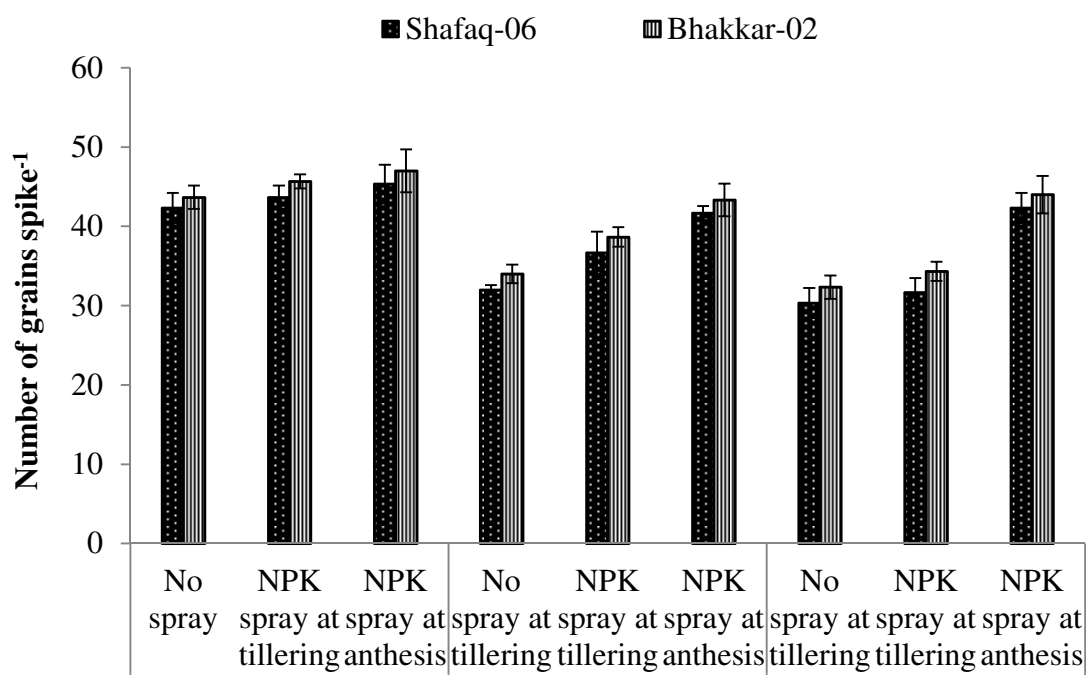


Fig. 4.54: Effect of supplemental foliar NPK application on number of grains spike⁻¹ of two wheat genotypes under different water levels during 2011-12 (mean values $\pm$ S.E).

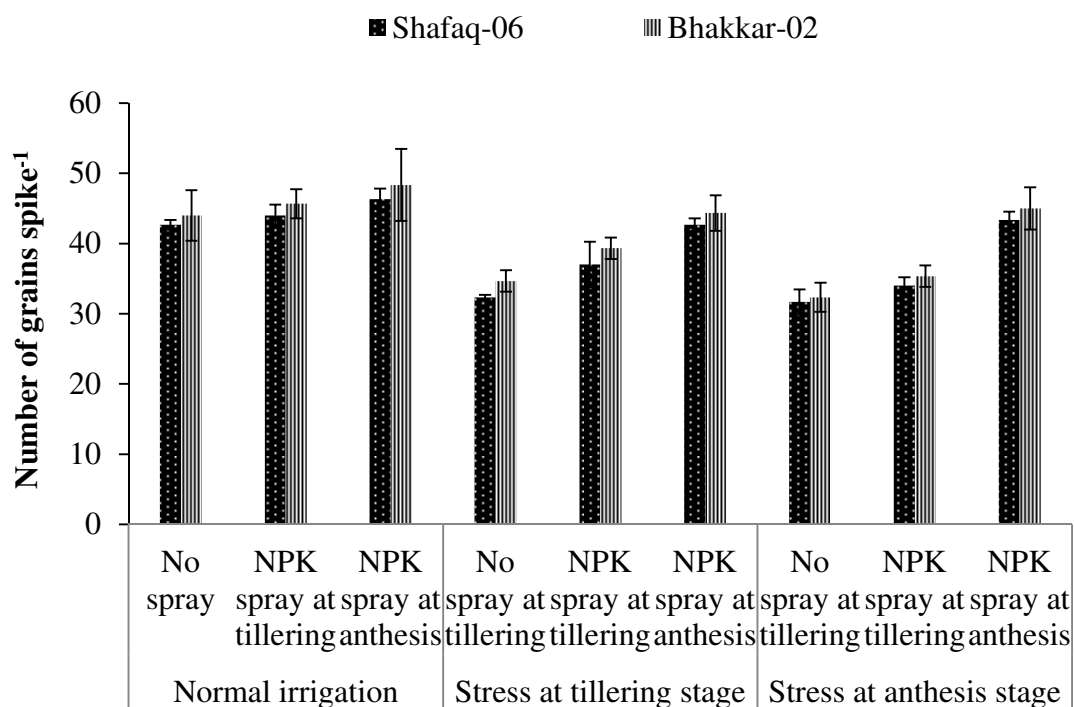


Fig. 4.55: Effect of supplemental foliar NPK application on number of grains spike⁻¹ of two wheat genotypes under different water levels during 2012-13 (mean values $\pm$ S.E).

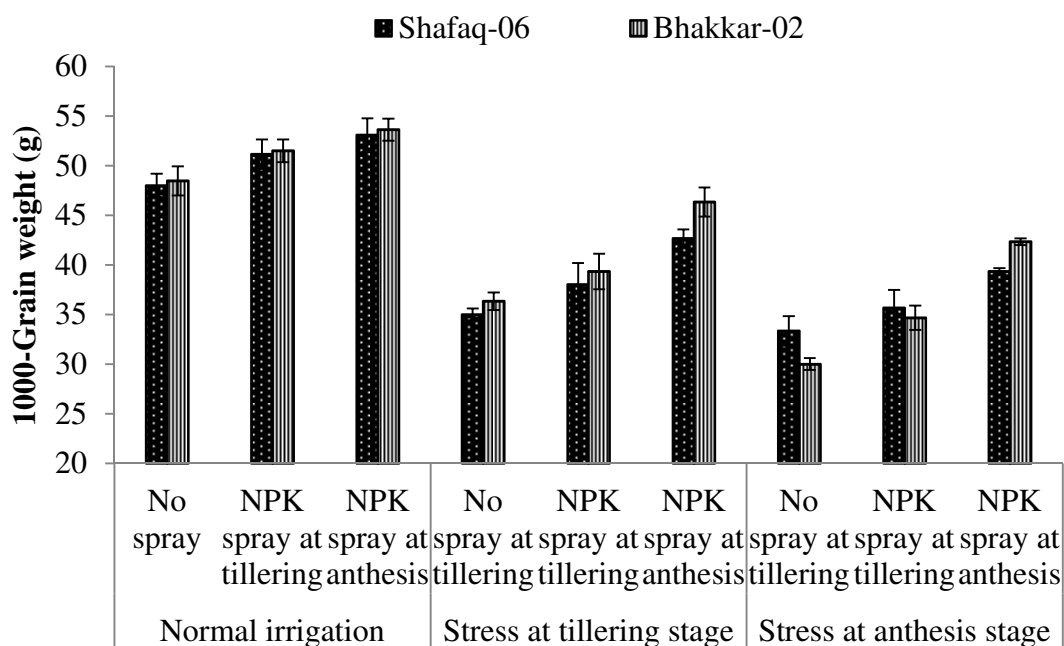


Fig. 4.56: Effect of supplemental foliar NPK application on 1000-grain weight (g) of two wheat genotypes under different water levels during 2011-12 (mean values $\pm$ S.E).

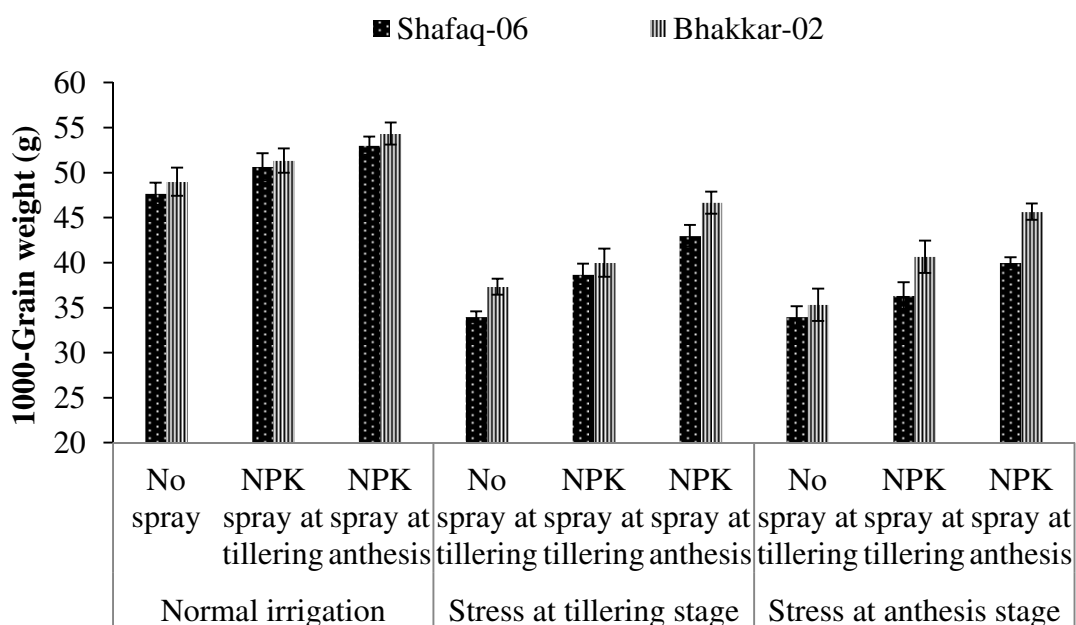


Fig. 4.57: Effect of supplemental foliar NPK application on 1000-grain weight (g) of two wheat genotypes under different water levels during 2012-13 (mean values $\pm$ S.E).

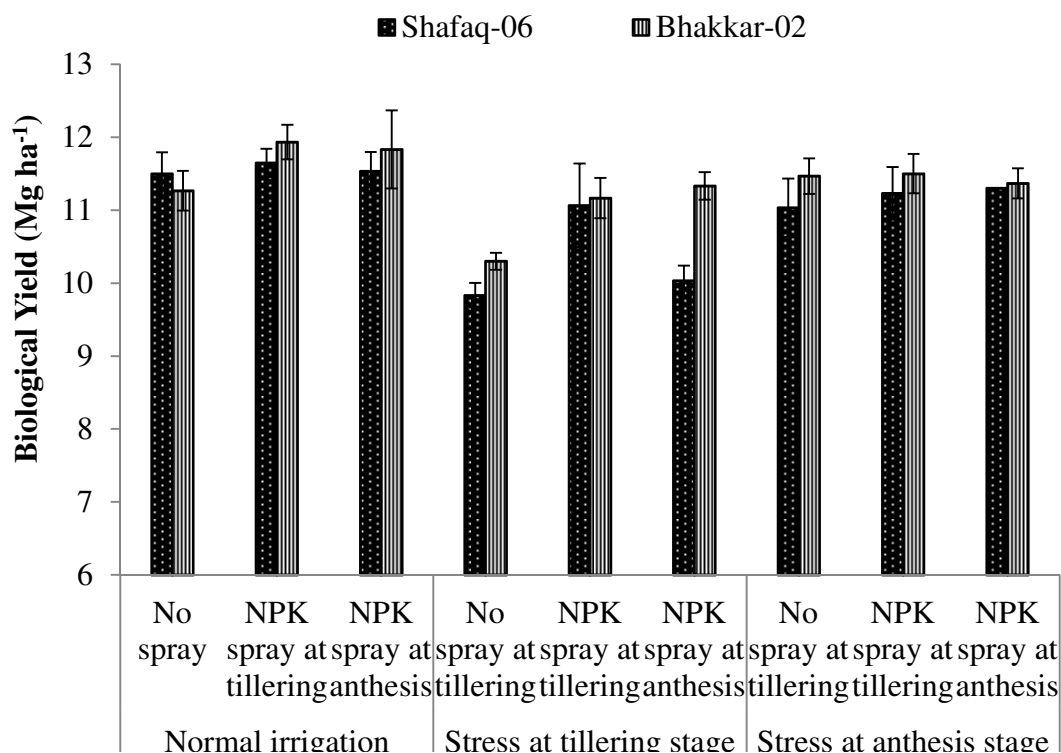


Fig. 4.58: Effect of supplemental foliar NPK application biological yield (Mg ha⁻¹) of two wheat genotypes under different water levels during 2011-12 (mean values $\pm$ S.E).

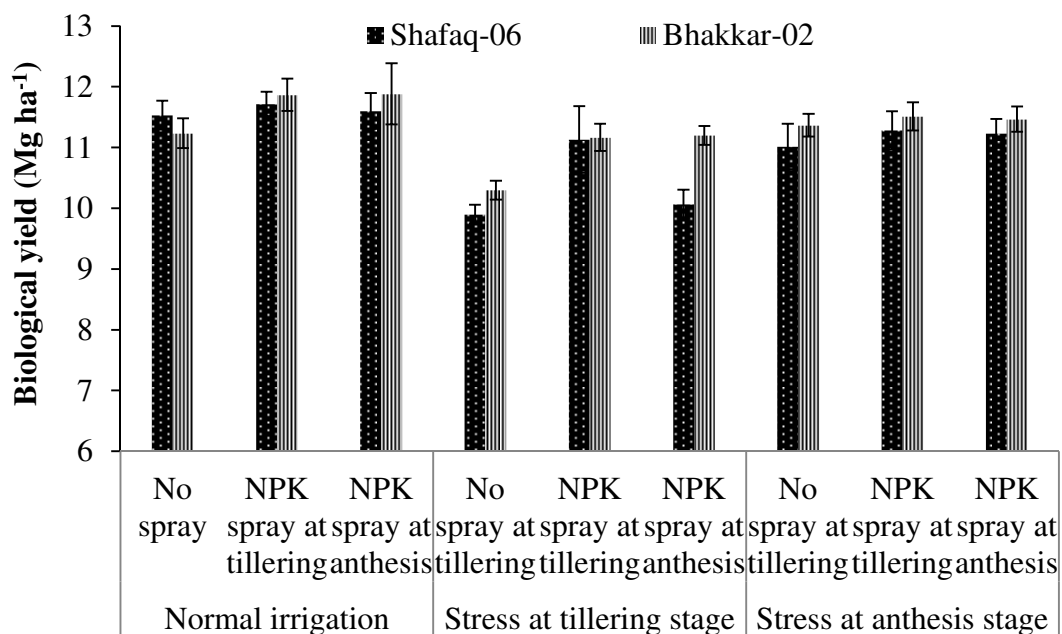


Fig. 4.59: Effect of supplemental foliar NPK application on biological yield (Mg ha⁻¹) of two wheat genotypes under different water levels during 2012-13 (mean values $\pm$ S.E).

4.5.9. Grain Yield

The grain yield was significantly ($P<0.001$) decreased under water limited conditions in both wheat genotypes during the year 2011-12 and 2012-13 (Table 4.11a, b). The reduction in grain yield was more pronounced in water limited plants at anthesis stage of growth. During 2011-12 and 2012-13, the drought stress at tillering and anthesis stage decreased grain yield by 19% and 23% respectively while during the second year (2012-13), reduction was 16% and 22% respectively as compared to normally irrigated plants (Appendix 4.55). Wheat genotype Bhakkar-02 produced only 4% higher grain yield as compared to Shafaq-06 in 2011-12 and 2012 -13 (Figs. 4.60, 4.61).

Highly significant difference for foliar NPK spray was observed for this variable. In 2011-12, plants foliarly sprayed with NPK at tillering and anthesis stage increased grain yield 9% and 13%, (4.03 Mg ha^{-1}), (4.44 Mg ha^{-1}) as compared to no spray (control) plants (3.87 Mg ha^{-1}) during 2011-12. In 2012-13, foliar NPK sprayed plants at tillering and anthesis stage increased grain yield 9% and 14%, (4.05 Mg ha^{-1}), (4.42 Mg ha^{-1}) as compared to no spray (control) plants (3.79 Mg ha^{-1}), (Appendix 4.55). All interactions were non-significant for grain yield during both years (Table 4.11a, b).

4.5.10. Harvest Index

Water stress significantly decreased ($P<0.001$) the harvest index during 2011-12 and 2012-13 respectively (Table 4.11a, b). In 2011-12, the drought stress conditions at tillering and anthesis stage significantly decreased it by 11% and 21% respectively while during the second year (2012-13), reduction was 8% and 20% respectively as compared to normally irrigated plants (Appendix 4.56). Wheat genotypes were non-significant for harvest index in 2011-12 and 2012-13 respectively (Figs. 4.62, 4.63).

The effect of foliar NPK spray treatment was also highly significant ($P<0.001$) for this variable. The foliar NPK spray at anthesis stage gave the maximum value (39.51%), (39.33) and minimum value (35.26%), (34.90%) was recorded in no spray treatment during 2011-12 and 2012-13 respectively (Appendix 4.56), (Figs. 4.62, 4.63). All other interactions were non-significant during both years (Table 4.11a, b).

Table 4.11a, b: Analysis of variance table for grain yield (Mg ha⁻¹) and harvest index (%) of two wheat genotypes in well-watered and water stress conditions with foliar applied nutrients NPK.

a.

SOV	Grain yield (Mg ha⁻¹)	Harvest index (%)
Genotypes (G)	*	NS
Water levels (W)	***	***
Treatments (T)	***	***
GXW	NS	NS
GXT	NS	NS
WXT	NS	NS
GXWXT	NS	NS
CV ^a	6.51	7.59

b.

SOV	Grain yield (Mg ha⁻¹)	Harvest index (%)
Genotypes (G)	*	NS
Water levels (W)	***	***
Treatments (T)	***	***
GXW	NS	NS
GXT	NS	NS
WXT	NS	NS
GXWXT	NS	NS
CV ^a	6.59	7.27

*, **, *** = Significant at 0.05, 0.01 and 0.001 level respectively

NS = Non significant; ^a coefficient of variation.

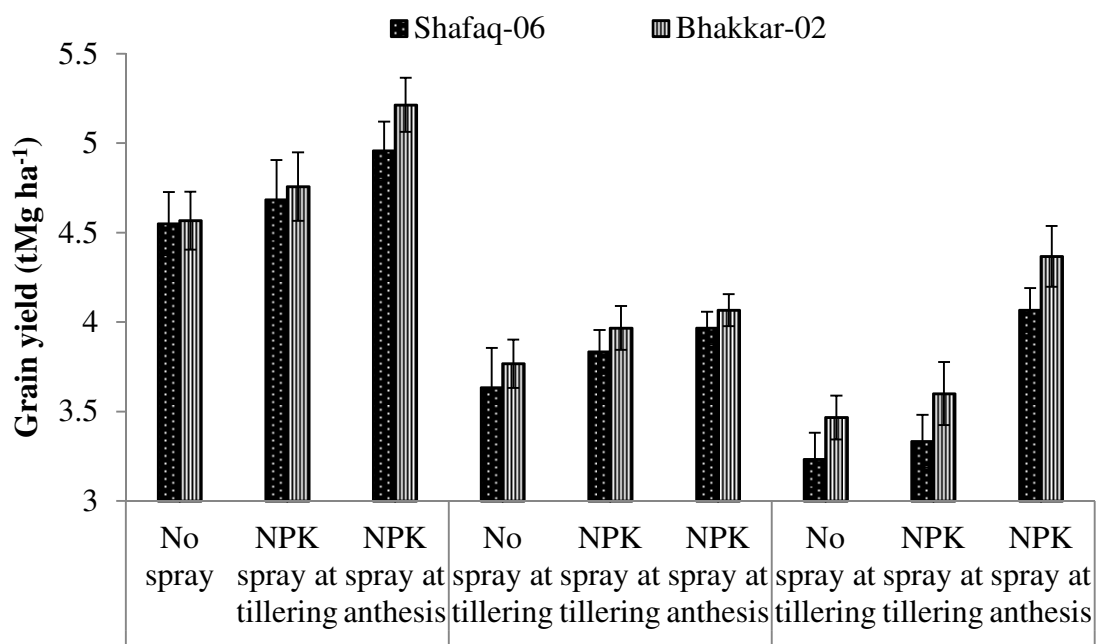


Fig. 4.60: Effect of supplemental foliar NPK application in combination on grain yield (Mg ha^{-1}) of two wheat genotypes under different water levels during 2011-12 (mean values $\pm$ S.E).

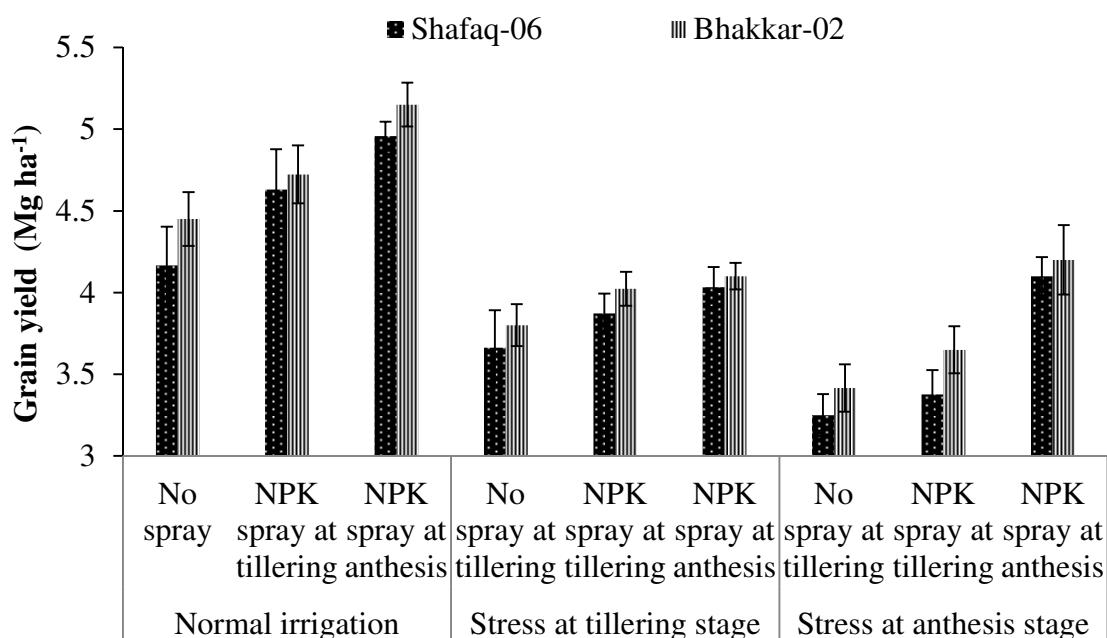


Fig. 4.61: Effect of supplemental foliar NPK application in combination on grain yield (Mg ha^{-1}) of two wheat genotypes under different water levels during 2012-13 (mean values $\pm$ S.E).

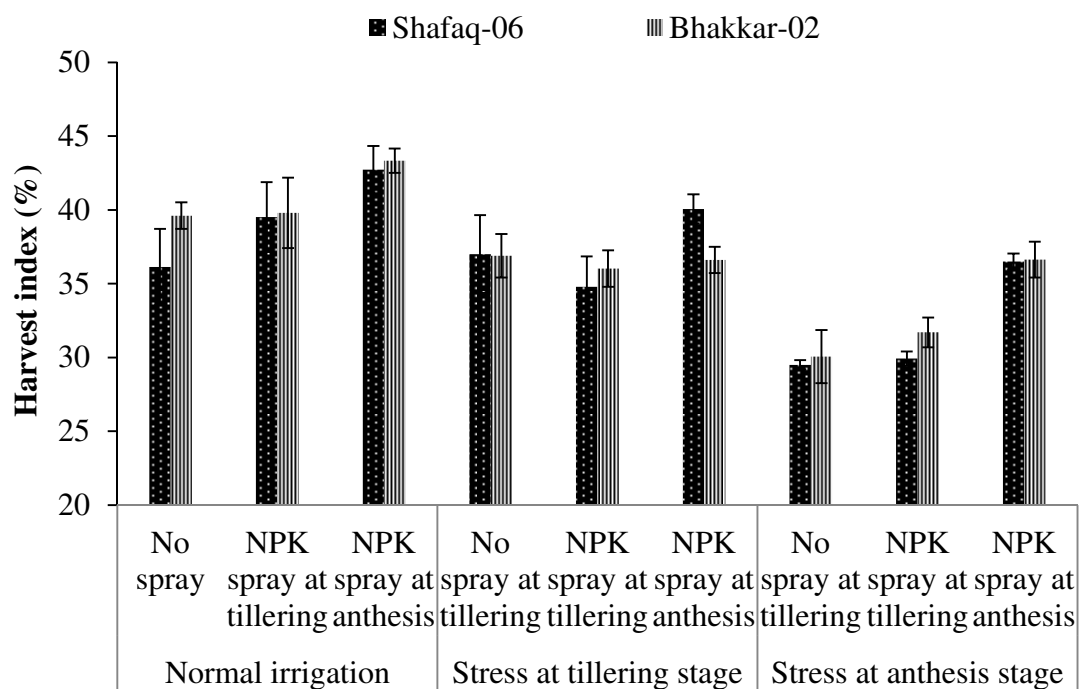


Fig. 4.62: Effect of supplemental foliar NPK application on harvest index (%) of two wheat genotypes under different water levels during 2011-12 (mean values $\pm$ S.E).

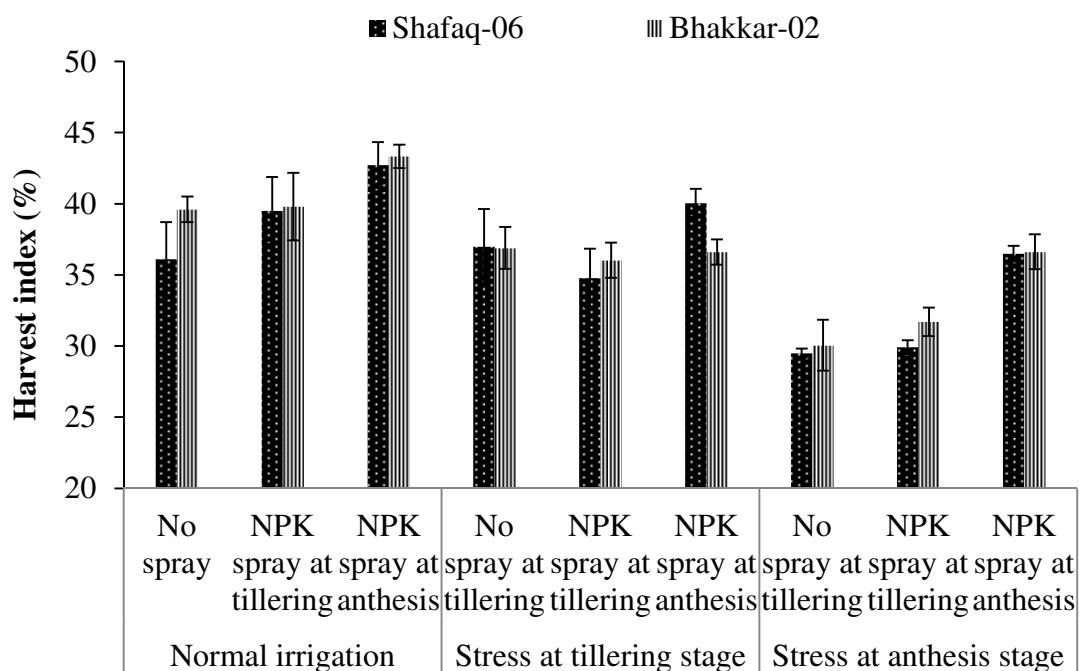


Fig. 4.63: Effect of supplemental foliar NPK application on harvest index (%) of two wheat genotypes under different water levels during 2012-13 (mean values $\pm$ S.E).

Chapter-5

DISCUSSION

Drastic reductions and worsening projections for water availability call for designing efficient crop management practices, particularly for staple food crops like wheat. Water-limitations adversely affect crop growth and development due to unfavourable conditions such as moisture and nutrient unavailability. Supplemental foliar fertilisation is one of the options to improve plant performance under sub-optimal environmental conditions. This study aims at improved understanding of physiological, biochemical, and morphological attributes of wheat in response to imposed water-stress and supplemental foliar fertilisation with basic macronutrients. To achieve the target objectives, a systematic experimental approach was adopted. In this combined approach, a series of experiments were conducted which involved: a) studies on germination and seedling growth in petri dishes and pots, b) optimisation of dose and combination of N, P, and K for foliar spray in glasshouse, c) identification of appropriate stage and physiological and biochemical attributes in response to optimised foliar spray in wire house, and d) finally, testing yield and yield components in response to optimised foliar spray under field conditions. In this final chapter, key findings of all conducted experiments are synthesised and discussed in the light of available literature on this topic. Main conclusions based on this study are presented in the last section.

5.1. Screening of Wheat Genotypes/Lines

For germination, the significant interaction between genotypes and PEG-induced water stress showed that the tested genotypes in experiment 1 were different in regards to germinability under different levels of water stress. Blum *et al.* (1980) also reported a large genetic variation among wheat genotypes in response to water stress maintained by using different osmotic (PEG) concentrations. Our results showed that PEG-induced water stress increased the time to germination and decreased the final germination percentage. The increase in PEG concentrations caused a decrease in the uptake of water by seeds, which resulted in declined germination percentage (Kaydan and Yagmur, 2008).

Germination stress index (GSI) can be used as an effective criterion for genotype screening against varying water stress levels (Fernandez, 1992). We calculated GSI values by using promptness index values of stressed and non-stressed (control) seeds. Our results showed that GSI decreased due to PEG-induced water stress irrespective of the genotype. Among tested genotypes, Bhakkar-02 recorded the maximum values of GSI at all levels of water stress. High values of GSI indicate the potential for drought tolerance (Zahra and Farshadfar, 2011).

Observations at the seedling stage in experiment 2 indicated that water stress induced after germination caused an extension in root length in all tested genotypes. This extension in root length happened at the cost of reduction in shoot length, thus shifting the relative root: shoot (R: S) equilibrium in favour of the roots, also reported by Larson, (1992), Ashraf and Sarwar, (2002), and Guoxiong *et al.* (2002). The increase in R:S length under water limitations may be attributed to reduction in supply of water and nutrients to the shoot. The possible explanation for reduction in shoot length under water-limited conditions might be the decrease in cell expansion, which ultimately reduces the plant height (Bajji *et al.*, 2000; Okçu *et al.*, 2005; Shahbaz *et al.*, 2011). Moreover, plant hormones can also play their role in the extension of root length under water-limited conditions (Sharp and Davis, 1985), which is particularly important to avoid drought stress in dry soils (Dhanda *et al.*, 1995). However, this may not be true for absolute root biomass which may increase in plants grown under well-watered conditions (Sharp and Davis 1985; Thornley, 1998).

Root length and seedling dry weight can be used as major selection criteria for screening genotypes against drought stress (Leishman and Westoby, 1994; Al-Karaki, 1998). Among tested genotypes, the drought-tolerant Bakhar-02 achieved maximum root length, root length stress index (RLSI), and total seedling dry weight whereas drought-sensitive Shafaq-06 had the least root length, RLSI, and total seedling dry weight. Deep roots and ability to accumulate higher biomass are considered typical characteristics of drought tolerant genotypes. Germination rate and final germination percentage correlate with root length and how much biomass is accumulated per unit area (Khan *et al.*, 2002; Ghodsi, 2004; Okçu *et al.*, 2005; Rauf *et al.*, 2007; Yamur and Kaydan, 2008).

5.1.1. Stress Indices and Foliar Applied Nutrients

Water stress affected the plant growth negatively. It is evident from our results of experiment 3 that supplemental foliar application of NPK in combination increased the plant height, root length, dry matter, net CO₂ assimilation rate, stomatal conductance, transpiration rate, water potential, osmotic potential, and turgor potential in two genotypes of wheat (Bhakkar-02 and Shafaq-06) under both well-watered and water-stress conditions. The nutrient content increased due to foliar fertilisation under water-stress conditions, which might have helped plants to cope with the negative effects of water stress on morphological and physiological characteristics as discussed in later parts of this discussion chapter. For example NPK application helped increasing plant height as a result of increase in the concentration of nutrients. Foliar application of NPK in combination recorded the maximum plant height under water stress conditions as compared to water spray and alone application of N, P, K, or in different combinations of two nutrients. The role of K in increasing plant height was reported earlier in wheat (Mesbah, 2009), barley (Aziz *et al.*, 2004), and maize (Anton and Ahmed, 2001). In general, the morpho-physiological response to supplemental foliar fertilisation was limited to treatment combinations in which K was included.

Roots are affected by water stress because root can move away from water in the soil due to shrinkage of root cells in drought-sensitive genotypes. Foliar application of NPK in combination increased the RLSI. The role of P and K in promoting root growth is well established. Under water-limited conditions, P increased the early root growth (Noack *et al.*, 2010) and K increased the root weight (Baque *et al.*, 2006) and root length (Saxena, 1985). High root density plays crucial role in obtaining water from the deeper soil layers whereas deeper roots decrease the moisture loss in soil. The promotion of root growth might increase water and nutrient uptake by plants (Rama Rao, 1986; Umar and Moinuddin, 2002). In addition to the role of P and K in promoting root growth, the use efficiency of N might also be indirectly affected.

Dry matter stress index decreased under water stress conditions but foliar application of nutrients also played crucial role in alleviating the impact of water stress on dry matter. Foliar application of NPK in combination helped in maintaining the dry matter stress index in a better way as compared to all other treatments possibly by supplementing the plant's requirement for macronutrients- N, P, and K. Increase in plant dry weight (root and shoot) with foliar spray of K₂SO₄ was reported in mung bean (Ihsan

et al., 2013) and wheat (Imanparsat *et al.*, 2013) and with foliar spray of KH_2PO_4 in tomato by Kaya *et al.* (2001).

5.2. Physiological Parameters

5.2.1. Gas Exchange Parameters

Net CO_2 assimilation rate (P_n), transpiration rate (E), and stomatal conductance (g_s) improved with supplemental foliar application of NPK spray, in both genotypes Bhakkar-02 and Shafaq-06 under water-limited and normal water supply. The increase in P_n , g_s and E rate was more pronounced with foliar NPK spray at tillering stage than at anthesis stage under both water conditions, but foliar NPK spray also improve these parameters at anthesis stage which might be more beneficial for the plant grain development under water-limited conditions. This also means that supplemental foliar fertilisation was also effective for improving the plant growth of drought-sensitive genotype. The decline in P_n under water stress may be associated with lower mesophyll capacity for net assimilation rate at the cellular level due to lower NPK accessibility for investment into photosynthetic apparatus. Nitrogen and K are involved in the regulation of net CO_2 assimilation rate in plants (Baker, 1996; Mengel and Kirkby, 2001).

Leaf nitrogen concentration decreased under water stress conditions which eventually lower the activity of rubisco and chlorophyll contents subsequently decreasing the P_n (Toth *et al.*, 2002). P_n increase with N application under water-limited conditions in maize (Zhang *et al.*, 2012), in winter wheat (Shangguan *et al.*, 2000). It is well documented that N improves the cell number and cell volume at cellular level and enhances the efficiency of P_n (Lawlor *et al.*, 1988). The stomatal conductance was more in high N plants of wheat as compare to N deficient plants (Li *et al.*, 2004). As with N application, increase in stomatal conductance with the application of P (Brück *et al.*, 2000), and K (Hu and Schmidhalter, 2005) in water-limited conditions. Stomatal regulation is an early reaction of plants to drought stress to reduce leaf E and maintain cell water status for biochemical activity, but that also leads to restriction of carbon uptake. The decrease in photosynthesis under water stress is primarily due to stomatal closure which restricts the carbon uptake by leaves (Cornic and Massacci, 1996). Optimal K-fertilisation is concomitant with increasing crop growth because of the positive effect of this nutrient in osmotic adjustment, stomatal regulation, photosynthesis, and protein synthesis (Ashraf and Naz, 1994; Quintero *et al.*, 1998). Potassium has been shown to

play a significant role in the opening and closing of leaf stomates which control the movement of CO₂ into the plant and water out into the air, and would therefore, have an effect on *g_s* (Bednarz *et al.*, 1998). Potassium application improved the *P_n*, *E* and intercellular CO₂ concentration under water stress conditions (Zhu *et al.*, 2012). Phosphorus under mild water deficit improved water use efficiency in P-treated wheat plants (dos-Santos *et al.*, 2004). The encouraging effects of P on plant growth under water stress have been ascribed as to enhancing the efficiency of *P_n*, *g_s*, and water use (Ackerson, 1985), to effects on water relations, and to higher cell membrane stability (Sawwan *et al.*, 2000).

5.2.2 Water Relations

Foliar application of NPK negatively affected the water relation parameters under water-limited (60% FC) conditions as compare to normal water supply (100% FC). The decrease in water potential, osmotic potential, turgor potential and relative water contents was less in foliar NPK spray under water-limited and normal water conditions. The foliar application of NPK maintained the water potential and osmotic potential and relative water contents at both tillering and anthesis stages under water-limited conditions in both wheat genotypes, Bhakkar-02 and Shafaq-06. The turgor potential was significantly improved by foliar NPK spray at anthesis stage. This shows that foliar spray of NPK in combination helped in maintaining the water status of plants possibly through osmotic adjustment, the accumulation of organic and inorganic ions such as free amino acids, proline, and K (Shabala and Lana, 2011).

Osmotic adjustment helps to retain a higher water level within the cells and thereby maintaining turgor under water stress conditions (Turner *et al.*, 2007). It is reported that decrease in leaf water potential and increase in bulk modulus elasticity (i.e a ratio of normal stress to a change in volume) together with decrease in osmotic potential maintain the plant turgor potential (Saito and Terashima, 2004). Water potential decreased with increases in leaf K concentration of plant through maintaining the turgor pressure in sunflower (Bajehbaj *et al.*, 2009). Potassium increased the water content and plants showed tolerance to water stress (Thalooth *et al.*, 2006). Ratnayaka and Kincaid, (2005), in contrast, reported non-significant differences in leaf water potential in senna plants with or without foliar-applied N under non-stress and stress conditions.

5.3. Biochemical

5.3.1. Chlorophyll Contents

The pigment which is responsible for photosynthesis in leaves is chlorophyll. Many studies showed a superior effect of environmental stress on the intensity of these pigments. Decrease in photosynthetic pigments production is an early symptom of drought stress (Ranjbarfordoei *et al.*, 2000), which primarily limits photosynthesis besides other effects of water stress such as closure of stomata and metabolic destruction (Lawson *et al.*, 2003). In the present study photosynthetic pigments in the leaves of wheat genotypes reduced under water stress. Drought stress diminished the chlorophyll “b” more than chlorophyll “a”. As a result, total chlorophyll concentration decreased as the water stress increased. Many studies specified a larger effect of environmental stress on the intensity of these pigments. Correspondingly, a decline in chlorophyll contents by dehydration, particularly in the older leaves was reported by David *et al.* (1998).

The photosynthetic pigments improved with the foliar applied NPK and the maximum chlorophyll “a”, chlorophyll “b”, and total chlorophyll contents were observed at tillering with foliar applied NPK in combination under normal conditions in drought tolerant genotype Bhakkar-02. Increase in chlorophyll pigments in the leaves by foliar K application was earlier reported by Suwanarit and Sestapukdee, (1989) and N-limitations frequently decreased the chlorophyll contents (Toth *et al.*, 2002). Low chlorophyll contents alter the leaf photosynthetic performance that may be due to the differences in plant N supply (Just *et al.*, 1989). Foliar application of N enhances chlorophyll a/b ratio, carotenoid content, and the total chlorophyll contents. Moreover, N boosts up the consistency of the chlorophyll, protein lipid complex, and photosynthetic activity. These findings are in line with the findings of Mouris *et al.* (1998) in maize. Application of K increased the chlorophyll contents in sunflower (Bajehbaj *et al.*, 2009). Ben-Dakhil *et al.* (2011) reported an increase in chlorophyll a content in potato by foliar K fertilisation in semi-arid conditions.

5.3.2. Total Soluble Sugars

Sugars act as osmotic solutes in fully and partly expanded leaves of wheat (Song, 2009). In the current study, a considerable increase in total soluble sugars in the leaves of both wheat genotypes Bhakkar-02 and Shafaq-06s was observed under water stress treatments. The outcome of these results are correlated to some previous finding by

Irigoyen *et al.* (1992), who also found that total soluble sugars enhanced due to water stress in alfalfa; they recommended the positive role of soluble sugars in osmoregulation under sub-optimal environment. It was observed throughout the current experiment that the sugar levels were higher in drought tolerant wheat genotypes. Munns and weir, (1981) have also described comparable results in wheat grown under water stress. The significant effect of foliar NPK application on total soluble sugars in the leaves of both wheat cultivars was observed in the present study and maximum total soluble sugars contents were obtained under water stress conditions with foliar applied NPK. Khan and Naqvi, (2012) reported that drought tolerant species have more accumulation of reducing sugars than in the drought sensitive species. So of the level of reducing sugar could be useful for the selection of drought tolerant species.

Limited water can affect a marked decrease in sucrose, fructose, and glucose contents of grains in sensitive wheat genotypes (Saeedipour, 2011). Kerepesi and Galiba, (2000) concluded that drought tolerant genotypes, in comparison with drought sensitive genotypes, accumulated additional sucrose. Couée *et al.* (2006) suggested that the accumulation of soluble compounds may be a tolerance strategy related with ROS-scavenging pathways for endurance under stress conditions. Total soluble sugars increased due to different environmental stresses in different parts of plants (Prado *et al.*, 2000; Gill *et al.*, 2001). Under conditions of combined salt and water stress (Gill and Singh, 1985; Prado *et al.*, 2000), sugar levels proliferate due to the increased intensity or duration of the stress. Plant metabolic procedures are dependent upon the adequate supply of N (Lawlor, 2002), which enhances the enzymes concerned in the synthesis of carbohydrates and sugars. The rise in leaf starch concentration due to the application of N was investigated earlier by Kettlewell *et al.* (1992) in wheat. Under abiotic stress conditions, sugars play an important part throughout plant growth and development by regulating carbohydrate metabolism. Stimulation of a huge amount of stress responsive genes by glucose has also been found, showing the part of sugars in environmental reactions (Price *et al.*, 2004).

5.3.3. Total Soluble Proteins

The decline in soluble proteins in the plants grown under drought stress condition was observed which is associated with the decreased rate of protein biosynthesis and proliferation in breakdown of proteins under water stress conditions (Rodriguez *et al.*, 2002). Total soluble proteins are essentially required in large amounts for osmotic

adjustment or osmoregulation (Nayyar and Walia, 2003). Foliar NPK application improved the leaf total soluble proteins contents with the increase in N and K concentration and maximum leaf soluble proteins were noted at foliar NPK application in combination in our experiment. These results are in accordance with previous findings of Mehrabdi and Mahassel, (2000) who found the increase in protein contents by foliar N (up to 1%) application in corn. The total soluble proteins increased with N application as soil amendment, e.g. in soybean (*Glycine max* L.) and wheat (Kettlewell *et al.*, 1992). Protein contents decreased in water stress conditions, so protein contents being susceptible to water stress are an indicator of water stress (Kattimani *et al.*, 1996). Grain protein contents increased with N application in wheat under water limited conditions (Pierre *et al.*, 2007; Stanciu and Neacsu, 2008). Potassium application also increased the protein contents in wheat under water stress conditions (Olgun *et al.*, 2006). The effect of K on plant may be due to its effect on enzymatic activity and role in the transportation and restoration of protein synthesis (Abd EL- Latif *et al.*, 2011). Rahim *et al.* (2010) reported increased in protein content through P-fertilisation.

5.3.4. Total Free Amino Acids

The role of amino acids in osmotic adjustment of plants grown under water stress conditions is well documented (Braam *et al.*, 1997; Dijksterhuis and De Vries, 2006). In the current study, a highly significant increase was observed in total free amino acids of water stressed plants of both wheat cultivars. An increase in total free amino acids under stressed environment was also reported by Ashraf and Iram, (2005). The escalation in total free amino acids due to water deficit can be attributed to reduction in total soluble proteins in wheat cultivars. Under water stress conditions, structural proteins break down into component amino acids, which take active part in osmotic adjustment under such stressful conditions (Good and Zaplachinski, 1994).

In this study significant effects of supplemental foliar applied NPK in combination on total free amino acids was observed under both water-limited (60% FC) and normal water supply (100% FC). The maximum leaf total free amino acids were observed at foliar NPK application in combination under 60% field capacity. The role of N in protein synthesis is well established. The escalation in breakdown of proteins under water stress conditions was reported by (Rodriguez *et al.*, 2002) ensuing in production of greater concentration of amino acids useful in osmoregulation (Nayyar and Walia, 2003). The N supply is essential for synthesis of proteins under water deficit conditions.

Atanasova, (2008) reported an increase in total free amino acids by N application. Optimal N and K availability is compulsory to sustain the growth under normal and stressed conditions because it is the main constituent of the hormones, enzymes and protein. The variations among the both cultivars for the accumulation of total free amino acids might be due to their genetic makeup (Rhodes and Samaras, 1994).

5.3.5. Nitrite and Nitrate Reductase Activity

Nitrate (NiR) and nitrite reductase (NR), the first enzymes in the pathway of N assimilation, have received due attention. The activity of these two enzymes has been reported to be reduced in water-stressed leaves of a variety of crop species. These enzymes participate in a crucial process involving uptake and translocation of NO_3 within and between the cells (Jackson *et al.*, 1986). Results of our study showed that the activity of NiR and NR decreased due to water deficit. Azedo-Silva *et al.* (2004) also reported changes in behaviour of NO_3 assimilatory enzymes in plants under water-stress conditions. Decrease in enzymatic activities under water deficit may be due to the decline in N and K availability under water stress conditions. Foliar application of N, P, and K, in our study, enhanced the activity of NiR and NR reductase under both normal and water-stressed conditions.

The highest activity was observed at anthesis in response to foliar applied NPK in combination under both normal and water-stressed conditions. The increased activity of NiR and NR under water stress conditions in response to N application has also been reported by Kathju *et al.* (1990). Foliar N fertilisation as a supplement source to soil-applied N is mostly proficient (Bondada *et al.*, 1997) for increasing N content of leaves and NiR, NR activities. Differences in drought tolerant and drought sensitive genotypes regarding NiR and NR may be due to their genetic makeup or accessibility of substrate as reported by Chen *et al.* (2004). As the activity of NiR and NR was studied under similar environmental conditions with similar concentration of substrate, so the decline in the present case may be due to decrease in enzyme synthesis. Potassium is known to be high mobile nutrient in plants. Potassium activates 60 enzymes related to plant growth (Marschner, 2002). Potassium showed positive effect on NiR activity in sunflower and safflower (Jabeen and Ahmad, 2011).

5.3.6. Proline Accumulation

The enhancement in leaf proline content was observed under water stress conditions in the current study. Enhancement in leaf proline content in response to abiotic stresses has been well documented (Ozturk and Demir, 2002; Hsu *et al.*, 2003; Ashraf and Foolad, 2007). In plants, a high concentration of proline helps to endure and adapt the sub-optimal environment (Nayyar and Walia, 2003). The proline content is commonly used as an indicator for the environmental stress in plants (Claussen, 2005; Gunes *et al.*, 2008). The process of proline accumulation in water stressed plants is possibly a result of decreased protein biosynthesis (Cechin *et al.*, 2006). The total soluble protein contents of plant grown in water-limited (60% FC) were significantly lower than those of normal water supply (100% FC). Therefore, high accumulation of proline in the leaves of 60% field capacity wheat plants was correlated with relatively low concentration of soluble proteins. The leaf proline contents improved with the foliar spray of NPK in combination. Related results were also documented in other studies where application of N (Monreal *et al.*, 2007) or K (Olgun *et al.*, 2006) enhanced the proline content in plant. Genetic make-up is accountable for differences in the accumulation of proline in wheat plants in the present study, also reported previously by Rhodes and Samaras, (1994). Therefore, production of proline in plants can be used as a criterion for drought stress resistance assessment for varieties.

5.3.7. Antioxidant Enzymes

Water stress in plants stimulates the production of ROS like singlet oxygen, hydrogen peroxide, super-oxides, and hydroxyl radicals (Jung, 2004). These ROS are highly reactive to membrane lipids, proteins, and DNA; hence these are believed to contribute as major factor for cellular damage (Yong *et al.*, 2006). Mechanism for the protection against ROS involved the several functionally interrelated antioxidant enzymes such as catalase, peroxidase, superoxide dismutase, and ascorbate peroxidase (Niedzwiedz-Siegien *et al.*, 2004). Water stress increased the activity of antioxidant enzymes (Salekjalali *et al.*, 2012). In present study, foliar application of NPK increased the activity of antioxidants including catalase, peroxidase, and ascorbate peroxidase under stress conditions. Increase in antioxidant enzymes is the most efficient mechanism against oxidative stress (Farooq *et al.*, 2009). Drought tolerant wheat genotypes produced high amount of ascorbate peroxidase and catalase under water stress condition as compared to drought sensitive ones (Sairam *et al.*, 1998). Application of N increased the antioxidant

activity of plant. The N nutrition had significant effect of catalase and peroxidase activity in *Catharanthus roseus* seedling (Misra and Gupta, 2006). Lu *et al.* (2012) also reported increased in catalase activity by N application before anthesis in maize seedlings. The application of K increase the superoxide dismutase, catalase, and glutathione peroxidase activity in sunflower under water stress conditions (Soleimanzadeh *et al.*, 2010).

5.4. Nutrients

5.4.1. Nitrogen Contents

Leaf N concentration decreased with the increase in water stress levels. Nitrogen concentration was significantly lower in the leaves of water stressed plants as compared to non-stressed plants in both wheat genotypes. However, foliar application of NPK increased the N concentration in both well-watered and water stress conditions. This finding corresponds to the findings of Morgan, (1986) for wheat, Satyanarayanaamma *et al.* (1996) for mungbean, Foyer *et al.* (1998) for maize, and Saneoka *et al.* (2004) for *Agrostis palustris* Huds.

Afifi *et al.* (2011) reported increase in N,P and K contents with supplemental foliar application of urea in maize. Murillo-Amador *et al.* (2006) reported that foliar application of calcium nitrite little bit increased the plant growth and nutrient concentration of cow pea under stressed conditions. N application showed positive effects on plant growth (Sadras, 2004). Maintained turgor of plants under water stress acquired with an optimal nutrient supply results in better growth of roots and subsequently promoted overall plant growth in maize plants (Studer *et al.*, 2007).

Apart from lowering nutrient availability in soil, plant uptake of nutrient very low might also be attributed to decreased the transpiration rate, which ultimately reduced the transport of nutrients from roots to shoots (Tanguilig *et al.*, 1987), caused the nutrient deficiency in plants, then the efficacy of foliar fertilisation became higher. The reasons for this are because of the supply of the required nutrient directly to the location of demand in the leaves and its relatively quick absorption (e.g. 0.5–2 h for N and 10–24 h for K), and independence of root activity and soil water availability (Römheld and El-Fouly, 1999).

5.4.2. Phosphorus Contents

Like N, the concentration of P decreased with the increase in water stress levels. The P concentration was significantly lower in the leaves of water stressed plants than those of non-stressed plants. The decline in NPK concentration of water-stressed plants can be described by the fact that under water-limited conditions, diffusion rate and mass flow of nutrient from rhizo-sphere to the root surface becomes slow due to the replacement of water by air in the soil pores (Chapin, 1991), ensuing in less availability of these nutrients to the plants. Hence, the transport of P from root to the leaf reduced due to less availability of this nutrient to the root. That is why low accumulation of P in water stressed plants of wheat genotypes was observed in our study, which is in line with the findings of Ashraf *et al.* (1998) for wheat. The diffusion coefficient of P in soil is very low, hence the root zone P is depleted and plants cannot acquire P when it is needed most (Clarkson, 1981).

Therefore, the utilisation of P as a foliar application becomes increasingly important. The mechanistic processes by which foliar applied nutrients are taken up are through leaf stomata (Eichert and Burkhardt, 1999) and hydrophilic pores within the leaf cuticle (Tyree *et al.*, 1990). From the present experiment results, it is clear that, under water stress conditions, foliar application of NPK in combination was beneficial in enhancing the leaf P concentration. Earlier reports suggest an increase in leaf P following foliar N sprays (Satyanarayananma *et al.*, 1996; Ashraf *et al.*, 2001). Hussein *et al.* (2013) reported increased in P contents with foliar application of K under water stress conditions in barley. However, our results are different in the sense that combined application of NPK as a foliar spray was more effective in enhancing P concentration than alone application of P as foliar spray.

5.4.3. Potassium Contents

Unlike N and P, leaf K concentration improved in both wheat genotypes under water stress. Highest K concentration was observed with foliar NPK spray in combination. The increase in tissue K concentration in response to water stress was reported in eggplant (Kirnak *et al.*, 2001), maize (Premachandra *et al.*, 1991), wheat (Baque *et al.*, 2006), and broccoli (Yildirim *et al.*, 2007). It is also reported that the increase in leaf K concentration results in a parallel increase in the stomatal conductance (Kant and Kafkafi, 2006). With an increase in leaf K, leaf osmotic potential reduced in this study. The rise in K concentration in stressed plants contributed to reducing leaf

osmotic potential (Morgan, 1992; Patakas *et al.*, 2002). Ouda *et al.* (2005) reported that the application of Potassium-P under water stress conditions proved to be effective in increasing N, P and K percentages in barley grains in all tested varieties. El-Kholy *et al.* (2005) emphasized that the highest N was recorded for barley cultivars (Giza123, 124, 125, 126, 129 130 and 2000) grown under normal conditions. P, K, and Na in all cultivars decreased under water stress condition. Foliar application of N, P, K and S at the four–five-leaf stages significantly increased the N and P contents of maize seedlings and resulted in an increased final grain yield (Giskin and Efron, 1986).

5.5. Yield and Yield Components

Water stress causes growth retardation (Baser *et al.*, 2004), consequently affecting the yield and yield components. An obvious reduction in spike length, number of spikelets per spike, 1000-grain weight, biological yield and grain yield under water stress conditions for both wheat genotypes was observed. Foliar application of NPK in combination at tillering or anthesis stages positively affected the yield parameters. Foliar application of N (Khan *et al.*, 2009), P (dos Santos *et al.*, 2004), and K (Mesbah, 2009) increased the grain yield of wheat. Under water stress conditions, nutrient uptake by roots is limited and plant leaves were symptomatic of nutrient deficiency, which in turn affects protein synthesis, cell structures, enzymatic activity, and metabolism. At organ level, in such conditions, plants may have less and smaller leaves (Fricke *et al.*, 1997), which is the main site of photosynthesis. All these damaging effects of reduced nutrient supply besides other foremost effects of drought stress on plant growth and development could be responsible for decline in yield and yield components.

Application of foliar NPK in combination to water stressed wheat plants helped to mitigate the negative effects of water limitations by improving several plants physiological and metabolic processes, therefore ultimately improving the yield and yield components. Many researchers also describe the mitigating water stress effects of nutrients in many crops. Application of combined NPK fertilizers resulted in the greatest grain yield, largest grain number, and grain weight (Liu, 2011). Burns and Ebelhar, (2006) reported that corn yield and grain weight increased with N fertilisation but not with K fertiliser on soils with high K in a cotton-corn rotation. In contrast, Amal *et al.* (2011) recommended that foliar K and N spray increased the plant height, number of spikes m⁻², weight of grains and biological yield in sandy soil. Baque *et al.* (2006) reported that dry matter accumulation was negatively affected by water stress in leaf,

root, and stem which was due to lower uptake of N, P, and K. Through the application of K in high amount, increase the concentration of N, P, and K occurred, particularly under water stress conditions. So, K application in wheat under water stress conditions might alleviate the adverse effects of water stress on wheat yield.

Benbella and Paulsen, (1998) showed that foliar applications after anthesis of 5 to 10 kg $\text{KH}_2\text{PO}_4 \text{ ha}^{-1}$ (1.1 to 2.2 kg P ha^{-1}) increased wheat grain yields by up to 1 Mg ha^{-1} . Applying P as foliar spray, in early growth stages, can increase the number of fertile tillers (Elliot *et al.*, 1997; Grant *et al.*, 2001). Mosali *et al.* (2006) identified Zadoks 32 (i.e. when second node is detectable during stem elongation) as the optimum time for foliar P spray as it increased both P-uptake and grain yield. Applications of P to wheat after anthesis increased grain yield (Benbella and Paulsen, 1998). Grain yields of corn positively responded to P at 2 kg P ha^{-1} applied as foliar spray from eighth leaf through to tasseling growth stages (Girma *et al.*, 2007). Mixed nutrient solutions have been widely tested (Garcia and Hanway, 1976; Alston, 1979; Ahmed *et al.*, 2006; Arif *et al.*, 2006,). Arif *et al.* (2006) investigated the effect of applying numerous foliar applications of mixed nutrient solutions (N, P and K) to wheat at tillering (Zadoks stage 26; main shoot and six tillers) and at booting (Zadoks stage 47; flag sheath opening) with one, two or three applications of the nutrient mix.

All treated sites produced higher grain yields as compared to the control (1695 kg ha^{-1}). Alston, (1979), Strong, (1982) and Gooding and Davies, (1992) also reported increase in grain yields with foliar applied N and P. According to Girma *et al.* (2007), only 50% of trials showed significant yield response to foliar P corresponding with sites with the lowest levels of initial soil P. Under water stress conditions, foliar P application had greater pod number and seed dry weight in bean (dos Santos *et al.*, 2004). Mosali *et al.* (2006) also suggested possible increases in grain yield, which resulted from foliar P spray, generally happened in seasons of water stress. This is most likely due to a reduced root-soil contact for exchange of nutrients, enhancing the benefits of foliar P in lower rainfall areas and/or years. Decreased in grain yield resulting from water limitation might be overcome by increasing K supply (Damon and Rengel, 2007). Foliar application of NPK increased the seed yield of lentil (*Lens culinaris* Medic) with basal NPK application as compared to alone application of N, P, and K (Humayun *et al.*, 2011).

5.6. Conclusion

Against the background of decreasing water availability and the need for cultivars, able to withstand water-limited environments, we set up screening experiments testing ten modern wheat genotypes. Additionally, we investigated the possible role of supplemental foliar fertilisation in ameliorating the water stress effects in early plant growth stages. Based on the germination parameters (in experiment 1) and stress indices (in experiment 2), we screened out two genotypes: Bhakkar-02 as a drought tolerant one and Shafaq-06 as a drought sensitive one. Next, we optimised the dose and combination of foliar sprays of nutrients, both under well-watered and water-stress conditions. We found that foliar spray NPK in combination (urea 1.5%, KH_2PO_4 2%, K_2SO_4 3%) treatment was the most effective in terms of improving plant growth, not only under well-watered conditions but also under water-stress conditions though to a lesser extent. This means that supplemental foliar fertilisation with foliar sprays containing three basic macronutrients (NPK) can help correcting nutrient deficiencies in water-limited environments where nutrient uptake is generally limited.

SUMMARY

The present study comprised of different experiments conducted in four phases to investigate the i) effect of water stress on various physiological and biochemical attributes of wheat, ii) effect of exogenously applied nitrogen, phosphorus and potassium on growth, yield, physiological and biochemical traits of wheat grown under drought conditions, iii) to determine optimum combination of supplemental foliar applied nitrogen, phosphorus, and potassium for improving drought tolerance and yield of wheat.

Water-limited conditions at early growth stages affect germination and seedling development, often leading to poor stand establishment. In phase 1 germination and seedling growth of ten local wheat genotypes viz. Lasani-08, Shafaq-06, Ufaq-06, Chakwal-86, Farid-06, Miraj-06, Manthar-03, Bhakkar-02, Faisalabad-08 and V₀-4178 in response to PEG induced water stress conditions (PEG-6000) and by withholding water supply were investigated. Two laboratory experiments were conducted to observe germination parameters and to calculate stress indices as screening criteria for drought tolerance. In first laboratory experiment, ten wheat genotypes were tested against PEG induced water stress of -0.2, -0.4, -0.6 and -0.8 MPa to screen out drought tolerant and drought sensitive genotypes. In tested genotypes, germination parameters viz. germination percentage, germination index, promptness index, and germination stress tolerance index declined in response to the increasing PEG-induced stress levels. In second laboratory experiment, the same ten wheat genotypes were grown in two sets of plastic pots; one set was watered at 100% field capacity whereas water was withheld in the other set of plastic pots at 8 to 28 days after sowing. Water stress conditions imposed by withholding irrigation at seedling stage reduced plant height stress tolerance index and dry matter stress tolerance index but increased root length stress tolerance index and relative root: shoot ratio. Based on results of germination parameters and stress indices, Bhakkar-02 was the most drought tolerant and Shafaq-06 was the most drought sensitive among tested ten genotypes.

In second phase, a pot experiment was conducted in glass house to evaluate the possible role of supplemental foliar fertilisation (optimise dose and combination) with macronutrients (NPK) at seedling stage. One drought tolerant, (Bhakkar-02) and one drought sensitive, (Shafaq-06) genotype as screened out in phase-1 were used in this study. Supplemental foliar fertilisation of macronutrients (NPK) alone or in different combinations improved the water relations, gas exchange parameters, and nutrient contents in both genotypes, (Bhakkar-02 and Shafaq-06). Foliar spray of NPK in combination was the most effective in improving plant growth under both well-watered and water-stress conditions. This best combination was further tested in wire house and field studies.

In phase 3, a pot experiment was conducted in wire house, in which the most effective NPK combination as found in phase 2, was evaluated to find out the most appropriate time (or stage) of supplemental foliar NPK application. In this experiment water relation, gas exchange and biochemical parameters were recorded. The water-stress (60% FC) conditions significantly decreased the water relations, gas exchange, and protein contents in both wheat genotypes i.e Shafaq-06 and Bhakkar-02. The water stress affected the anthesis stage more severely as compared to tillering stage. The foliar application of NPK in combination significantly improved the nutrient (N, P and K) concentration in the leaves of both genotypes which enhanced the accumulation of osmolytes (proline, soluble sugars, and total free amino acids) and the activity of antioxidants- catalase, peroxidase, and ascorbate peroxidase under water-stress conditions. However, foliar application of NPK in combination was more effective at anthesis stage as compared to tillering stage in water stress conditions.

In phase 4, two field experiments were conducted during growing seasons 2011/12 and 2012/13 to estimate the effect of supplemental foliar NPK spray on yield and yield components of wheat genotypes exposed to water deficit conditions. During both years a significant decrease in yield and yield components was recorded in water-stressed plants at both stages i.e tillering and anthesis. The water stress at anthesis stage decreased the yield and its components more severely. The foliar application of NPK improved the number of grains per spikelet and 1000-grain weight under both well-watered and water stress conditions. The foliar application of NPK was the most effective at anthesis stage under both well-watered and water-stress conditions.

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Appendices

Appendix 4.1: Effect of supplemental foliar NPK application on net CO₂ assimilation rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	11.90 b	12.86 ab	7.07 def	7.85 de	5.73 f	7.06 def	6.13 ef	6.91 def	8.19 B
NPK spray	12.12 ab	13.84 a	8.18 cd	9.97 c	6.90 def	7.84 de	6.55 def	7.16 def	9.07 A
WL means	10.47 A				6.49 B				

Appendix 4.2: Effect of supplemental foliar NPK application on net CO₂ assimilation rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	9.262 b	10.398 a	9.83 A
Anthesis	6.883 d	7.972 c	7.43 B
Genotypes Means	8.07 B	9.19 A	

Appendix 4.3: Effect of supplemental foliar NPK application on stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	4.92bc	5.20 b	4.21 d	4.46 cd	2.39 fg	2.46 efg	2.09 g	2.30 g	3.50 B
NPK spray	5.07 bc	6.09 a	4.95 bc	5.90 a	2.57 efg	2.99 ef	2.45 efg	3.05 e	4.13 A
WL means	5.10 A				2.54 B				

Appendix 4.4: Effect of supplemental foliar NPK application on stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	3.74 bc	4.19 a	3.96 A
Anthesis	3.42 c	3.93 ab	3.68A
Genotypes Means	3.58 B	4.06 A	

Appendix 4.5: Effect of supplemental foliar NPK application on transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	4.13b	4.26 c	3.08 de	3.22 d	1.73 g	1.88 g	1.92 g	2.02 g	2.78 B
NPK spray	5.66 a	5.92 a	4.06 c	4.80 b	2.45 f	2.90 e	2.37 f	3.13 de	3.91 A
WL means	4.39 A				2.30 B				

Appendix 4.6: Effect of supplemental foliar NPK application on transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	3.50 b	3.74 a	3.62 A
Anthesis	2.86 d	3.29 c	3.08 B
Genotypes Means	3.18 B	3.52 A	

Appendix 4.7: Effect of supplemental foliar NPK application on water potential (- MPa) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	- 0.86 c	- 0.78 b	- 0.98 ef	- 0.92 d	- 1.157 h	- 1.08 g	- 1.42 l	- 1.32 j	- 1.07 B
NPK spray	- 0.80 b	- 0.72 a	- 0.88 c	- 0.80 b	- 1.0 f	- 0.95 e	- 1.36 k	- 1.19 i	- 0.96 A
WL means	-0.84 A				-1.19 B				

Appendix 4.8: Effect of supplemental foliar NPK application on water potential (- MPa) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	-0.95 b	0.89 a	9.83 A
Anthesis	-1.16 d	-1.06 c	7.43 B
Genotypes Means	-1.06 A	-0.97 A	

Appendix 4.9: Effect of supplemental foliar NPK application on osmotic potential (- MPa) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	- 1.26 b	- 1.20 a	- 1.51 d	- 1.51 d	- 1.35 c	- 1.35 c	- 1.66 f	- 1.65 f	- 1.44 A
NPK spray	- 1.25 b	- 1.20 a	- 1.54 de	- 1.56 e	- 1.25 b	- 1.25 b	- 1.75 g	- 1.66 f	- 1.43 A
WL means	-1.38 A				-1.49 B				

Appendix 4.10: Effect of supplemental foliar NPK application on osmotic potential (- MPa) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	-1.28 b	-1.25 a	9.83 A
Anthesis	-1.62 d	-1.59 c	7.43 B
Genotypes Means	-1.45 A	-1.42 A	

Appendix 4.11: Effect of supplemental foliar NPK application on turgor potential (MPa) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	0.40 efg	0.42 ef	0.54 cd	0.58 bc	0.20 i	0.27 hi	0.24 hi	0.33 fgh	0.37 B
NPK spray	0.46 de	0.48 de	0.66 ab	0.75 a	0.25 hi	0.30 gh	0.40 efg	0.46 de	0.47 A
WL means	0.54 A				0.31B				

Appendix 4.12: Effect of supplemental foliar NPK application on turgor potential (MPa) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	0.33 c	0.37 c	0.35 B
Anthesis	0.46 b	0.53 a	0.50 A
Genotypes Means	0.39 B	0.45 A	

Appendix 4.13: Effect of supplemental foliar NPK application on relative water contents (%) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	84.67 ab	93.62 a	83.96 ab	85.29 ab	66.40 de	80.88 abc	64.80 e	69.29 cde	78.62 B
NPK spray	93.67 a	93.33 a	85.97 ab	87.30 ab	78.82 bcd	90.10 ab	67.59 de	83.52 ab	85.04 A
WL means	88.48 A				75.18 B				

Appendix 4.14: Effect of supplemental foliar NPK application on relative water contents (%) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	80.89 b	89.48 a	85.19 A
Anthesis	75.58 b	81.35 b	78.47 B
Genotypes Means	78.24 B	85.42 A	

Appendix 4.15: Effect of supplemental foliar NPK application on chlorophyll (a) (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	1.37 bcde	1.44 abcd	0.98 gh	1.02 fgh	1.26 def	1.31 cde	0.81 h	0.99 fgh	1.15 B
NPK spray	1.51 abcd	1.57 abc	1.11 efg	1.14 efg	1.60 ab	1.66 a	0.91 gh	1.15 efg	1.32 A
WL means	1.27 A				1.21 A				

Appendix 4.16: Effect of supplemental foliar NPK application on chlorophyll (a) (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	1.43 a	1.49 a	1.46 A
Anthesis	0.95 b	1.07 b	1.01 B
Genotypes Means	1.19 A	1.28 A	

Appendix 4.17: Effect of supplemental foliar NPK application on chlorophyll (b) (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	0.64 cde	0.70 bcd	0.57efghi	0.63 def	0.57 fghi	0.60 efg	0.52 hi	0.53 ghi	0.60 B
NPK spray	0.70 bc	0.78 a	0.62 def	0.72 ab	0.50 i	0.62 ef	0.58 efgh	0.62 ef	0.65 A
WL means	0.67 A				0.57 B				

Appendix 4.18: Effect of supplemental foliar NPK application on chlorophyll (b) (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	0.60 bc	0.67 a	0.64 A
Anthesis	0.57 c	0.62 b	0.60 B
Genotypes Means	0.59 B	0.65 A	

Appendix 4.19: Effect of supplemental foliar NPK application on total chlorophyll (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	2.0 de	2.15 bcd	1.58 gh	1.67 g	1.85 ef	1.87 ef	1.34 i	1.51 h	1.75 B
NPK spray	2.19 bc	2.37 a	1.72 fg	1.85 f	2.11 cd	2.27 ab	1.50 hi	1.85 f	1.98 A
WL means	1.94 A				1.79 B				

Appendix 4.20: Effect of supplemental foliar NPK application on total chlorophyll (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	2.04 b	2.16 a	2.10A
Anthesis	1.54 d	1.72 c	1.63 B
Genotypes Means	1.79 B	1.94 A	

Appendix 4.21: Effect of supplemental foliar NPK application on total carotenoids (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	0.41 cdefg	0.42 bcdef	0.37 fg	0.42 bcdef	0.39 defg	0.43 abcde	0.35 g	0.42 bcdef	0.40 B
NPK spray	0.49 a	0.48 ab	0.43abcdef	0.43 abcde	0.44 abcd	0.46 abc	0.38 efg	0.48 ab	0.45 A
WL means	0.43 A				0.42 A				

Appendix 4.22: Effect of supplemental foliar NPK application on total carotenoids (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	0.44 a	0.45 a	0.44 A
Anthesis	0.38 b	0.44 a	0.41 B
Genotypes Means	0.41 B	0.44 A	

Appendix 4.23: Effect of supplemental foliar NPK application on total soluble sugars (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	0.97 j	2.85 fgh	3.24 def	3.82 bcd	1.81 i	2.40 hi	3.81 bcd	4.05 bc	2.87 B
NPK spray	2.54 gh	3.06 efg	3.65 cde	4.34 b	2.97 fgh	3.47 cdef	5.14 a	5.67 a	3.85 A
WL means	3.06 B				3.67 A				

Appendix 4.24: Effect of supplemental foliar NPK application on total soluble sugars (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	2.07 d	2.94 c	2.51 B
Anthesis	3.96 b	4.47 a	4.22 A
Genotypes Means	3.02 B	3.71 A	

Appendix 4.25: Effect of supplemental foliar NPK application on total soluble proteins (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	10.51 c	10.82 c	11.25 bc	12.0 ab	7.34 hi	8.0 fgh	6.64 i	8.33 efg	9.36 B
NPK spray	11.04 c	11.15 c	12.17 a	12.73 a	8.44 ef	8.91 de	7.58 gh	9.49 d	10.20 A
WL means	11.46 A				8.10 B				

Appendix 4.26: Effect of supplemental foliar NPK application on total soluble proteins (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	9.34 b	9.72 b	9.53 B
Anthesis	9.41 b	10.64 a	10.02 A
Genotypes Means	9.38 B	10.18 A	

Appendix 4.27: Effect of supplemental foliar NPK application on total free amino acid (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	15.05 h	17.29 gh	18.97 fg	19.32 efg	19.96 efg	20.4	23.41abcd	24.07 abc	19.79 B
NPK spray	15.28 h	18.98 fg	19.95 efg	21.20cdef	20.29 ef	21.97bcde	24.89 ab	26.03 a	21.16 A
WL means	18.26 B				22.70 A				

Appendix 4.28: Effect of supplemental foliar NPK application on total free amino acid (mg g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	17.82 c	19.63 b	18.72 B
Anthesis	21.81 a	22.65 a	22.23 A
Genotypes Means	19.81 B	21.14 A	

Appendix 4.29: Effect of supplemental foliar NPK application on nitrate reductase ($\mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	3.53 efgh	3.17 gh	3.17 gh	3.40 fgh	3.53efgh	2.09 i	2.88 h	2.92 h	3.09 B
NPK spray	4.50 bc	4.98 b	4.26 cd	5.93 a	3.91 cdef	4.37 bc	4.13 cde	3.65 defg	4.46 A
WL means	4.12 A				3.44 B				

Appendix 4.30: Effect of supplemental foliar NPK application on nitrate reductase ($\mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	3.87 ab	3.65 ab	3.76 A
Anthesis	3.61 b	3.98 a	3.79 A
Genotypes Means	3.74 A	3.81 A	

Appendix 4.31: Effect of supplemental foliar NPK application on nitrite reductase ($\mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	2.48 gh	3.14 fg	3.62 def	3.97 cd	1.94 hi	3.29 ef	1.74 i	2.22 hi	2.80 B
NPK spray	5.01 ab	5.48 a	3.93 cde	5.01 ab	3.10 fg	3.66 def	3.58 def	4.53 bc	4.29 A
WL means	4.08 A				3.01 B				

Appendix 4.32: Effect of supplemental foliar NPK application on nitrite reductase ($\mu\text{mol NO}_2 \text{ g}^{-1} \text{ FW h}^{-1}$) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	3.13 b	3.22 b	3.51 A
Anthesis	3.90 a	3.93 a	3.58 A
Genotypes Means	3.17 B	3.91 A	

Appendix 4.33: Effect of supplemental foliar NPK application on leaf proline contents (mmol proline g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	0.79 fg	0.65 g	2.15 cd	2.37 cd	0.92 fg	1.40 ef	2.28 cd	2.41 cd	1.62 B
NPK spray	0.95 fg	0.93 fg	2.31 cd	2.85 bc	1.21 efg	1.81 de	3.52 b	4.30 a	2.24 A
WL means	1.63 B				2.23 A				

Appendix 4.34: Effect of supplemental foliar NPK application on leaf proline contents (mmol proline g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	0.97 c	1.20 c	1.08 B
Anthesis	2.57 b	2.98 a	2.78 A
Genotypes Means	1.77 B	2.09 A	

Appendix 4.35: Effect of supplemental foliar NPK application on ascorbate peroxidase (ABA digested g⁻¹ FW h⁻¹) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	0.87 e	0.82 e	0.97 de	1.48 cd	0.79 e	0.92 e	2.59 b	2.71 ab	1.40 B
NPK spray	1.09 cde	1.19 cde	1.09 cde	1.60 c	0.85 e	1.29 cde	2.89 ab	3.13 a	1.64 A
WL means	1.14 B				1.90 A				

Appendix 4.36: Effect of supplemental foliar NPK application on ascorbate peroxidase (ABA digested g⁻¹ FW h⁻¹) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	0.90 c	1.06 c	0.98 B
Anthesis	1.89 b	2.23 a	2.06 A
Genotypes Means	1.39 B	1.65 A	

Appendix 4.37: Effect of supplemental foliar NPK application on catalase activity (units min⁻¹ g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	112 i	149 fgh	216 e	302 d	138 gh	153.0 fgh	320.67 cd	342 bc	216.58 B
NPK spray	132 hi	157 fg	331 bc	347 b	151 fgh	170.0 f	347 b	375 a	251.58 A
WL means	218.25 B				249.58 A				

Appendix 4.38: Effect of supplemental foliar NPK application on catalase activity (units min⁻¹ g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	133.25 d	157.25 c	145.25 B
Anthesis	303.67 b	341.50 a	322.58 A
Genotypes Means	218.46 B	249.38 A	

Appendix 4.39: Effect of supplemental foliar NPK application on peroxidase activity (units min⁻¹ g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	735.05 j	737.61 ij	747.27fghij	757.37efghi	739.49 hij	753.67efghij	758.48defgh	769.58 cde	749.81 B
NPK spray	742.93 ghij	763.43 def	769.58 cde	777.57 bcd	761.62defg	783.90 bc	791.79 b	827.42 a	777.29 A
WL means	753.86 B				773.24 A				

Appendix 4.40: Effect of supplemental foliar NPK application on peroxidase activity (units min⁻¹ g⁻¹ FW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	744.77 c	759.66 b	752.22 B
Anthesis	766.78 b	782.99 a	774.88 A
Genotypes Means	755.78 B	771.32 A	

Appendix 4.41: Effect of supplemental foliar NPK application on nitrogen contents (mg g⁻¹ DW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	27.10 c	33.36 ab	12.97 g	17.80 de	32.93 ab	32.80 ab	15.20 efg	17.87 de	23.75 B
NPK spray	31.27 b	33.73 ab	13.80 fg	20.20 d	34.63 ab	34.90 a	17.17 def	19,97 d	25.71 A
WL means	23.78 B				25.68 A				

Appendix 4.42: Effect of supplemental foliar NPK application on nitrogen contents (mg g⁻¹ DW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	31.48 b	33.70 a	32.59 A
Anthesis	14.78 d	18.96 c	16.87 B
Genotypes Means	23.13 B	26.33 A	

Appendix 4.43: Effect of supplemental foliar NPK application on phosphorus contents (mg g⁻¹ DW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	2.59 hi	3.99 bcd	4.01 bcd	4.59 b	2.25 i	3.84 cde	3.05 fgh	3.77 cde	3.51 B
NPK spray	3.29 efg	5.75 a	4.38 bc	6.02 a	2.66 ghi	4.04 bcd	3.41 def	4.09 bc	4.20 A
WL means	4.32 A				3.39 B				

Appendix 4.44: Effect of supplemental foliar NPK application on phosphorus contents (mg g⁻¹ DW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	2.70 c	4.41 a	3.55 B
Anthesis	3.71 b	4.61 a	4.16 A
Genotypes Means	3.20 B	4.51 A	

Appendix 4.45: Effect of supplemental foliar NPK application on potassium contents (mg g⁻¹ DW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Treatments	Well-watered (100% FC)				Water stress (60% FC)				Treatment means
	Tillering		Anthesis		Tillering		Anthesis		
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
No spray	25.13 h	27.87 gh	28.23 gh	30.54 fg	30.54 fg	36.07 cde	34.71 def	40.04 bc	31.64 B
NPK spray	29.18 gh	37.78 bcd	31.93 efg	34.71 def	32.39 efg	41.78 ab	41.78 ab	46.0 a	36.93 A
WL means	30.67 B				37.90 A				

Appendix 4.46: Effect of supplemental foliar NPK application on potassium contents (mg g⁻¹ DW) of two wheat genotypes in well-watered (100% FC) and water stress (60% FC) conditions.

Stages	Shafaq-06	Bhakkar-02	Stages means
Tillering	29.31 c	35.85 ab	31.64 B
Anthesis	34.15 b	37.82 a	35.98 A
Genotypes Means	31.73 B	36.84 A	

Appendix 4.47: Effect of supplemental foliar NPK application on number of tillers of two wheat genotypes under different water levels during 2011-12 and 2012-13.

	2011-12							2012-13						
	Normal irrigation		Stress at tillering		Stress at anthesis		Means	Normal irrigation		Stress at tillering		Stress at anthesis		Means
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02		Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
T₁	568.33bc	579.33bc	450 e	493.33 d	573.33bc	563.67 c	567.28 A	563 bcd	572 abc	446.33 e	480.33 e	567 abcd	553 cd	530.61B
T₂	595.33ab	581 bc	563.67 c	560 c	579.33bc	578.33bc	576.28 A	591.3ab	570 abc	556 bcd	550 cd	575abc	572 abc	569.22A
T₃	585 bc	578.33bc	570 bc	482.67 d	614 a	573.67bc	537.94 B	580abc	571 abc	531.67 d	473.33 e	600 a	561 bcd	553.22C
WL Means	581.22 A		519.94 B		580.33 A			574.89 A		506.39 B		571.78 A		

T₁= No spray; T₂= Foliar NPK spray at tillering stage; T₃= Foliar NPK spray at anthesis stage, WL= Water levels

Appendix 4.48: Effect of supplemental foliar NPK application on number of fertile tillers of two wheat genotypes under different water levels during 2011-12 and 2012-13.

	2011-12							2012-13						
	Normal irrigation		Stress at tillering		Stress at anthesis		Means	Normal irrigation		Stress at tillering		Stress at anthesis		Means
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02		Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
T₁	415 efgh	448.3 abc	306.67 k	313.33 k	390 hij	406.67 fgh	380 C	408.33 def	443.33 ab	299 i	308.33 i	400 ef	403 ef	377 B
T₂	445 Abcd	453.33 ab	376.67 ij	394.33 ghi	435.67 bcde	438.33 bcde	423.9 A	440.33 abc	448.33 ab	366.67 gh	387 fg	435.67 abc	431.67 abcd	418.28 A
T₃	436.67 bcde	467.33 a	421.67 cdef	364 j	407.67 fgh	418.33 defg	419.3 C	421.67 bcde	458.33 a	400 ef	360 h	406 def	415 cde	410.17 A
WL Means	444.28 A		362.68 C		416.18 B			436.72 A		353.50 C		415.22 B		

T₁= No spray; T₂= Foliar NPK spray at tillering stage; T₃= Foliar NPK spray at anthesis stage, WL= Water levels

Appendix 4.49: Effect of supplemental foliar NPK application on plant height (cm) of two wheat genotypes under different water levels during 2011-12 and 2012-13.

	2011-12							2012-13						
	Normal irrigation		Stress at tillering		Stress at anthesis		Means	Normal irrigation		Stress at tillering		Stress at anthesis		Means
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02		Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
T₁	92.55 def	94.03 bcd	88.33 h	89.67 gh	90.63 fgh	92.33 defg	91.26 C	93.33 bcde	93.33 bcde	89.67 fg	88.67 g	90.67 efg	92 cdef	91.28 C
T₂	95.80 abc	98.17 a	91.15 efg	94.40 bcd	93 def	98.28 a	95.13 A	95 b	99.07 a	90.67 efg	93.73 bcd	93.67 bcd	98.70 a	95.14 A
T₃	96.37 ab	94 bcd	91.67 defg	91 efgh	91.86 defg	93.49 cde	93.07 B	95.67 b	94.48 bc	91 defg	89.66 fg	91.86 cdef	93.33 bcde	92.67 B
WL Means	95.15 A		91.04 C		93.27 B			95.15 A		90.57 C		93.37 C		

T₁= No spray; T₂= Foliar NPK spray at tillering stage; T₃= Foliar NPK spray at anthesis stage, WL= Water levels

Appendix 4.50: Effect of supplemental foliar NPK application on spike length (cm) of two wheat genotypes under different water levels during 2011-12 and 2012-13.

	2011-12							2012-13						
	Normal irrigation		Stress at tillering		Stress at anthesis		Means	Normal irrigation		Stress at tillering		Stress at anthesis		Means
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02		Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
T₁	11.3 bc	12.63 a	10.07 f	10.87 cde	11.2 bc	12.43 a	11.42 B	11.22 b	12.53 a	9.87 f	10.73 cde	11.25 b	12.42 a	11.36 B
T₂	11.59 b	12.71 a	10.75 de	11.59 b	11.1bcde	12.54 a	11.71 A	11.49 b	12.59 a	10.66 e	11.50 b	11.17 bc	12.40 a	11.63 A
T₃	11.44 b	12.65 a	10.13 f	10.67 e	11.16bcd	12.60 a	11.44 B	11.37 b	12.53 a	10 f	10.67 de	11.15 bcd	12.47 a	11.36 B
WL Means	12.05 A		10.68 C		11.85 B			11.95 A		10.57 B		11.81 A		

T₁= No spray; T₂= Foliar NPK spray at tillering stage; T₃= Foliar NPK spray at anthesis stage, WL= Water levels

Appendix 4.51: Effect of supplemental foliar NPK application on number of spikelets spike⁻¹ of two wheat genotypes under different water levels during 2011-12 and 2012-13.

	2011-12							2012-13						
	Normal irrigation		Stress at tillering		Stress at anthesis		Means	Normal irrigation		Stress at tillering		Stress at anthesis		Means
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02		Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
T₁	18.33 a	18.33 a	14.33 c	14.33 c	17.67 ab	17.67 ab	16.78 A	18.33 ab	18.33 ab	14.33 c	15 c	18.33 ab	18.33 ab	17.11 A
T₂	19 a	19 a	14.33 c	15.67 bc	18.33 a	18.33 a	17.44 A	19 a	18.33 ab	14.33 c	16.33 bc	18.33 ab	18.33 ab	17.44 A
T₃	19 a	19 a	14.33 c	15 c	18.33 a	17.67 ab	17.22 A	19.67 a	19 a	14.33 c	15 c	18.33 ab	18.33 ab	17.44 A
WL Means	18.78 A		14.67 B		18 A			18.78 A		14.89 B		18.33 A		

T₁= No spray; T₂= Foliar NPK spray at tillering stage; T₃= Foliar NPK spray at anthesis stage, WL= Water levels

Appendix 4.52: Effect of supplemental foliar NPK application on number of grains spike⁻¹ of two wheat genotypes under different water levels during 2011-12 and 2012-13.

	2011-12							2012-13						
	Normal irrigation		Stress at tillering		Stress at anthesis		Means	Normal irrigation		Stress at tillering		Stress at anthesis		Means
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02		Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
T₁	42.33 bc	43.67 ab	32 f	34 ef	30.33 f	32.33 ef	35.78 C	42.67 bc	44 b	32.33 f	34.67 ef	31.67 f	32.33 f	36.28 C
T₂	43.67 ab	45.67 ab	36.67 de	38.67 cd	31.67 f	34.33 def	38.44 B	44 b	45.67 ab	37 de	39.33 cd	34 ef	35.33 def	39.22 B
T₃	45.33 ab	47 a	41.67 bc	43.33 ab	42.33 bc	44 ab	43.94 A	46.33 ab	48.33 a	42.67 bc	44.33 ab	43.33 bc	45 ab	45 A
WL Means	44.61 A		37.72 B		35.83 C			45.17 A		38.39 B		36.94 B		

T₁= No spray; T₂= Foliar NPK spray at tillering stage; T₃= Foliar NPK spray at anthesis stage, WL= Water levels

Appendix 4.53: Effect of supplemental foliar NPK application on 1000-grain weight of two wheat genotypes under different water levels during 2011-12 and 2012-13.

	2011-12							2012-13						
	Normal irrigation		Stress at tillering		Stress at anthesis		Means	Normal irrigation		Stress at tillering		Stress at anthesis		Means
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02		Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
T₁	48 bc	48.47 bc	35 fg	36.33 efg	33.33 gh	30 h	38.52 C	47.67 de	49 cde	34 j	37.33 hij	34 j	35.33	39.56 C
T₂	51.15 ab	51.50 ab	38.03 ef	39.33 de	35.67 fg	34.67 fg	41.73 B	50.67 bcd	51.33 abc	38.67 hi	40 gh	36.33 ij	40.67 gh	42.94 B
T₃	53.07 a	53.63 a	42.67 d	46.33 c	39.33 de	42.33 d	46.23 A	53 ab	54.33 a	43 fg	46.67 e	40 gh	45.67 ef	47.11 A
WL Means	50.97 A		39.62 B		35.89 C			51.0 A		39.94 B		38.67 B		

T₁= No spray; T₂= Foliar NPK spray at tillering stage; T₃= Foliar NPK spray at anthesis stage, WL= Water levels

Appendix 4.54: Effect of supplemental foliar NPK application on biological yield (Mg ha⁻¹) of two wheat genotypes under different water levels during 2011-12 and 2012-13.

	2011-12							2012-13						
	Normal irrigation		Stress at tillering		Stress at anthesis		Means	Normal irrigation		Stress at tillering		Stress at anthesis		Means
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02		Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
T₁	11.50 ab	11.27 ab	9.83 d	10.30 cd	11.03 bc	11.47 ab	10.90 B	11.53 ab	11.23 ab	9.90 d	10.30 cd	11.02 bc	11.37 ab	10.89 B
T₂	11.65 ab	11.93 a	11.07 abc	11.17 abc	11.23 ab	11.50 ab	11.43 A	11.72 ab	11.87 a	11.13 ab	11.17 ab	11.28 ab	11.51 ab	11.45 A
T₃	11.53 ab	11.83 ab	10.03 d	11.33 ab	11.30 ab	11.37 ab	11.34 AB	11.60 ab	11.88 a	10.07 d	11.20 ab	11.23 ab	11.47 ab	11.24 A
WL Means	11.62 A		10.62 B		11.32 A			11.64 A		10.63 B		11.31 A		

T₁= No spray; T₂= Foliar NPK spray at tillering stage; T₃= Foliar NPK spray at anthesis stage, WL= Water levels

Appendix 4.55: Effect of supplemental foliar NPK application on grain yield (Mg ha⁻¹) of two wheat genotypes under different water levels during 2011-12 and 2012-13.

	2011-12							2012-13						
	Normal irrigation		Stress at tillering		Stress at anthesis		Means	Normal irrigation		Stress at tillering		Stress at anthesis		Means
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02		Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
T₁	4.55 bc	4.57 bc	3.63 efghi	3.77 efghi	3.23	3.47 ghi	3.87 B	4.17 ef	4.45 cde	3.66 ghij	3.80 fghi	3.25 j	3.42 ij	3.79 C
T₂	4.68 bc	4.76 bc	3.83 efg	3.97 def	3.33 hi	3.60 fghi	4.03 B	4.63 bcd	4.72 abc	3.87 fgh	4.02 efgh	3.38 ij	3.65 hij	4.05 B
T₃	4.96 ab	5.21 a	3.97 def	4.07 de	4.07 de	4.37 cd	4.44 A	4.96 ab	5.15 a	4.03 efghi	4.10 efg	4.10 efg	4.20 def	4.42 A
WL Means	4.79 A		3.87 B		3.68 C			4.68 A		3.92 B		3.67 C		

T₁= No spray; T₂= Foliar NPK spray at tillering stage; T₃= Foliar NPK spray at anthesis stage, WL= Water levels

Appendix 4.56: Effect of supplemental foliar NPK application on harvest index (%) of two wheat genotypes under different water levels during 2011-12 and 2012-13.

	2011-12							2012-13						
	Normal irrigation		Stress at tillering		Stress at anthesis		Means	Normal irrigation		Stress at tillering		Stress at anthesis		Means
	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02		Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	Shafaq-06	Bhakkar-02	
T₁	39.65 abcd	40.52 abc	37 cde	36.58 cde	29.29 h	30.30 gh	35.56 B	36.20 bc	39.60 ab	37.06 bc	36.92 bc	29.49 e	30.12 e	34.90 B
T₂	40.24 abc	39.95 abcd	34.82 efg	35.57 def	29.65 h	31.32 fgh	35.26 B	39.55 ab	39.91 ab	34.95 cd	36.06 bcd	29.90 e	31.70 de	35.35 B
T₃	43.06 ab	44.12 a	39.53 abcd	35.89 cdef	35.97 cde	38.49 bcde	39.51 A	42.80 a	43.40 a	40.07 ab	36.62 bc	36.49 bc	36.59 bc	39.33 A
WL Means	41.26 A		36.57 B		32.50 C			40.24 A		36.94 B		32.38 C		

T₁= No spray; T₂= Foliar NPK spray at tillering stage; T₃= Foliar NPK spray at anthesis stage, WL= Water levels